STUDY REPORT
Original: 1/2

STUDY TITLE

ACUTE INHALATION TOXICITY STUDY OF BIO-X KLEANZE EC IN SPRAGUE DAWLEY RATS

(As per OECD Guideline No. 403: Acute Inhalation Toxicity)

STUDY No.: BIO-ITX 259

Study Completion Date: 06 February 2021

SPONSOR

OKADA ECOTECH PTE LTD,
24 PIONEER CRESCENT #04-08
628557 SINGAPORE

TEST FACILITY

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CONFIDENTIAL

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QUALITY ASSURANCE STATEMENT

The Study No.: BIO-ITX 259, entitled "Acute Inhalation Toxicity Study of BIO-X Kleanze EC in Sprague Dawley Rats" has been inspected according to the OECD Principles of Good Laboratory Practice [C(97)186/Final].

The dates of inspections and dates of reporting to the Study Director and the Management have been listed below:

Inspection Dates	Inspection Phases	Reporting Dates	
		Study Director	Management
Initiation Phase			
14 December 2020	Study plan verification	14 December 2020	14 December 2020
29 January 2021	Study plan amendment no. 1 verification	29 January 2021	29 January 2021
In Life Phase			
28 December 2020	Test item exposure - sighting study	29 December 2020	29 December 2020
Reporting Phase			
28 January 2021	Draft report inspection	28 January 2021	28 January 2021
05 February 2021	Final report inspection	05 February 2021	05 February 2021

Inspections were performed according to the Standard Operating Procedures of the test facility's Quality Assurance Unit. The study report was inspected against the approved study plan and pertinent raw data and accurately reflects the raw data.

.....

(Signature)
Mr. PRAVEEN B.
Quality Assurance Unit

06 February 2021

(Date)

STATEMENT OF GLP COMPLIANCE

The Study No. BIO-ITX 259, entitled "Acute Inhalation Toxicity Study of BIO-X Kleanze EC in Sprague Dawley Rats" was performed in compliance with the OECD Principles of Good Laboratory Practice [C(97)186/Final].

DECLARATION

I hereby declare that the work was performed under my supervision and in accordance with the described procedures. It is assured that the reported results faithfully represent the raw data obtained during the experimental work. No circumstances have been left unreported which may have affected the quality or integrity of the data or which might have a potential bearing on the validity and reproducibility of this study.

I accept overall responsibility for the technical conduct of the study as well as the interpretation, analysis, documentation and reporting of the results.

K. Yella Subbadu
(Signature)
Mr. YELLA SUBBADU K.
Study Director

06 February 2021
(Date)

STATEMENT OF CONFIDENTIALITY

This report contains **CONFIDENTIAL** and **PROPRIETARY** information of **OKADA ECOTECH PTE LTD., SINGAPORE** and will not be disclosed to anyone without the expressed or written approval of sponsor, except to the employees of test facility wherever necessary and to persons authorized by law or judicial judgment.

K. Yella Subbadu
(Signature)
Mr. YELLA SUBBADU K.
Study Director

06 February 2021
(Date)

(Signature)
Dr. NITIN M. SHETTY
Deputy Test Facility Management

06 February 2021
(Date)

ABBREVIATIONS OF COMMONLY USED UNITS AND SYMBOLS

AAALAC	-	Association for Assessment and Accreditation of Laboratory Animal Care
B	-	Breadth
CPCSEA	-	Committee for the Purpose of Control and Supervision of Experiments on Animals
ECD	-	Effective Cut-off Diameter
F	-	Female
g	-	Gram
GHS	-	Globally Harmonized System of Classification and Labelling of Chemicals
GLP	-	Good Laboratory Practice
GSD	-	Geometric Standard Deviation
h/hr(s)	-	Hour(s)
H	-	Height
IAEC	-	Institutional Animal Ethics Committee
kg	-	Kilogram
L/min	-	Liter per minute
L	-	Length
M	-	Male
min	-	Minute
mg	-	Milligram
mg/L	-	Milligram/Liter
mL	-	Milliliter
mm	-	Millimeter
MMAD	-	Mass Median Aerodynamic Diameter
n	-	Number of animals
NAD	-	No Abnormality Detected
N	-	Normal
No.	-	Number
OECD	-	Organization for Economic Co-operation and Development
psi	-	pound per square inch
ppm	-	parts per million
TS	-	Terminal Sacrifice
SD	-	Standard Deviation
%	-	Percent
µm	-	Micrometer
°C	-	Degree Celsius

1. STUDY DETAILS

1.1	**Study Title**	**:**	Acute Inhalation Toxicity Study of BIO-X Kleanze EC in Sprague Dawley Rats
1.2	**Study Number**	**:**	BIO-ITX 259
1.3	**Study Code**	**:**	AITR
1.4	**Sponsor Details**		
	Sponsor	:	Okada Ecotech Pte Ltd, 24 Pioneer Crescent #04-08 628557 Singapore
	Sponsor's Representative and Monitoring Scientist	:	K. E. Tan Okada Ecotech Pte Ltd, 24 Pioneer Crescent #04-08 628557 Singapore
	Monitoring Scientist	:	A. Z. Tan Okada Ecotech Pte Ltd, 24 Pioneer Crescent #04-08 628557 Singapore
1.5	**Test Facility**	**:**	Bioneeds India Private Limited Devarahosahally, Sompura Hobli, Nelamangala Taluk, Bangalore Rural District, PIN - 562 111, Karnataka, India.
1.6	**Study Responsibilities**		
	Study Director	:	Mr. Yella Subbadu K., M.Sc. Bioneeds India Private Limited, Devarahosahally, Sompura Hobli, Nelamangala Taluk, Bangalore Rural District, PIN - 562 111, Karnataka, India E-mail: bioneeds@bioneeds.in
	Study Co-ordinator	:	Ms. Priyanka Sharma., M.Sc.
	Study Personnel	:	Mr. Koushik P., B. Tech. (Biotech).
	Study Veterinarians	:	Dr. K. R. Sneha., M.V.Sc.
	Study Pathologists	:	Dr. Prajapati Ramdatt Khemabhai., M.V.Sc.

1.7 Study Schedule

Study Initiation Date	:	18 December 2020
Experimental Starting Date	:	22 December 2020
Acclimatization Date	:	Start: 22 December 2020 End: 28 December 2020
Treatment Date	:	For G1: 28 December 2020 For G2: 29 December 2020
Necropsy Date	:	For G1: 11 January 2021 For G2 : 12 January 2021
Experimental Completion Date	:	12 January 2021
Draft Report Submission Date	:	31 January 2021
Study Completion Date	:	05 February 2021

2. SUMMARY

The test item, BIO-X Kleanze EC was evaluated for acute inhalation toxicity in Sprague Dawley rats.

The objective of the study was to assess the toxic potential and to determine the LC_{50} of test item, BIO-X Kleanze EC when administered by inhalation route through flow-past nose-only dynamic inhalation equipment for a single 4 hours exposure to rats. Six male and six female rats were used for study.

The Undiluted test item was used during technical pre-test and limit test to generate the liquid aerosols through collision nebulizer. The technical pre-test was carried out without animals. To achieve the maximum concentration in the limit test the air flow rate selected was 12 L/min with 7.7 L/min aerosol generator air and 4.3 L/min dilution air for G1 and 7.0 L/min aerosol generator air and 5.0 L/min dilution air for G2 based on the technical pretest results.

During Limit test, the particle size Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) were 2.90 µm to 3.13 µm and 2.61 to 2.62 respectively for G1 and 2.91 µm to 3.12 µm and 2.56 to 2.58 for G2 respectively. All the values were within the range. The mean maximum achievable breathing zone concentration (actual concentration) was 5.03 mg/L and 5.04 mg/L of air for G1 and G2 respectively and it was considered as the limit concentration.

All the animals were observed for clinical signs and pre-terminal deaths, during exposure and post-exposure on day 1 and once daily thereafter for clinical signs and twice daily for mortality till 14 days post exposure period. Individual animal body weight was recorded. All rats were euthanized after 14 days post exposure period by intraperitoneal administration of sodium thiopentone and the gross pathological findings were recorded.

No treatment related clinical signs of toxicity and mortalities were observed. Slight decrease in body weight was noted on day 2 due to exposure. All animals showed increase in body weight on day 4, 8 and 15.

No treatment related gross pathological findings were noted at the mean maximum achievable concentration of 5.03 and 5.04 mg/L of air for G1 and G2 respectively.

Conclusion

Under the experimental conditions employed and based on the above results of experiment, there were no clinical signs and mortality observed at mean maximum achievable concentration of 5.03 mg/L for G1 and 5.04 mg/L of air for G2. Hence, the LC_{50} of the test item, BIO-X Kleanze EC is >5.03 and 5.04 mg/L of air and is classified as “Category 5” according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

3. STUDY COMPLIANCE

3.1 GLP Compliance

The study was performed:

a. In compliance with the OECD Principles of Good Laboratory Practice [C(97)186/Final].

b. In accordance with the Standard Operating Procedures at Bioneeds India Private Limited and as per the mutually agreed study plan with the sponsor.

3.2 Regulatory Guideline

The study was performed in accordance with OECD Guidelines for Testing of Chemicals No. 403 - Traditional Protocol "Acute Inhalation Toxicity" adopted on 07 September 2009.

3.3 Animal Welfare

The study was performed in an AAALAC accredited facility:

a. In accordance with the recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animal facility published in the Gazette of India, 2018.

b. In accordance with the protocol approved by Institutional Animal Ethics Committee (IAEC) (Protocol No.: BIO-IAEC-4020, Date of Approval: 08 September 2020).

4. SAFETY PRECAUTIONS

Gloves, head cap, face mask and goggles were used in addition to the protective body garments and slippers to ensure adequate personnel health and safety and to avoid ingestion, inhalation, skin and eye contact with the test item.

5. OBJECTIVE

The objective of this study was to assess the toxic potential and to estimate the LC_{50} of test item BIO-X Kleanze EC when administered through nose-only inhalation route for single 4 hours exposure to rats and also to classify for risk assessment of chemical according to globally harmonized system of classification and labelling of chemicals.

6. MATERIALS AND METHODS

6.1 Test Item Information

The test item information provided by the sponsor as per Test Item Data Sheet and Certificate of Analysis is presented below:

Name of Test Item	:	BIO-X Kleanze EC
Physical appearance (with color)	:	Clear Brown Liquid
Batch No.	:	2020061201
Date of Manufacture	:	12 June 2020
Date of Expiry	:	12 June 2023
Storage Conditions	:	Ambient (21 to 29°C)

Batch Produced by (Name and address)	:	Okada Ecotech Pte Ltd, Singapore
Test Item Code by Test Facility	:	D1155-001

The responsibility for the correct identity and stability of the test item rests with the sponsor. The Certificate of Analysis of test item provided by sponsor is presented as Annexure 1.

6.2 Preparation of Test item

80 dilutions: 1 mL of test item was taken and 79 mL of distilled water was added to get 80 dilutions of test item.

160 dilutions: 0.5 mL of test item was taken and 79.5 mL of distilled water was added to get 160 dilutions of test item.

6.3 Test System

Animal Species : Rat (*Rattus norvegicus*)

Strain : Sprague Dawley

Justification for Selection of Species : Rat is the preferred laboratory rodent species for inhalation toxicity assessment and also recommended by the various regulatory guidelines.

Source of Supply : In-house bred animals

Body Weight Range at Receipt : Males : 185.59 g to 194.80 g
Females : 163.50 g to 174.66 g

No. of Animals/ Group and Sex : Sighting Study/Limit Test:- 3 Males and 3 Females/ Group

Group	Test Item	Animal No.	
		Males	Females
G1	BIO-X Kleanze EC (80 Times Dilution)	Rf0195 to Rf0197	Rf0198 to Rf0200
G2	BIO-X Kleanze EC (160 Times Dilution)	Rf0201 to Rf0203	Rf0204 to Rf0206

(Females used were nulliparous and non-pregnant)

Age at Treatment : 9-10 weeks

Animal Identification : During Acclimatization period: All the animals were identified by tail marking using a red permanent marker pen. Additionally, a cage card was displayed which included study no., cage no., sex, animal no. (temporary), start date and end date of acclimatization period.

During Treatment period: The animals were identified by writing last 4 digits of the animal number on the tail using a black permanent marker pen and additionally, a cage card was displayed which included study no., cage no., sex, dose, animal no. (permanent), treatment date and date of necropsy.

6.4 Husbandry

a. Environmental Conditions : Animals were housed under standard laboratory conditions, in an environmentally monitored air-conditioned room with adequate fresh air supply (12 to 15 air changes per hour), room temperature 19.3°C to 22.9°C and relative humidity 43% to 68%, with 12 hours fluorescent light and 12 hours dark cycle. The temperature and relative humidity were recorded once daily.

b. Housing : Three animals per sex were housed in a standard polypropylene cage (size: L 430 × B 285 × H 150 mm) with stainless steel mesh top grill having facilities for holding pelleted feed and drinking water in water bottle fitted with stainless steel sipper tube. Clean sterilized paddy husk was provided as bedding material.

c. Feed : Altromin maintenance diet 1324 for rats and mice (manufactured by Altromin Spezialfutter GmbH & Co. KG) was provided *ad libitum* to the animals throughout the experimental period (except during restraining and exposure period). The contaminant analysis test report of the feed is presented as Annexure 2.

d. Water : Water was provided *ad libitum* throughout the acclimatization and experimental period. Deep bore-well water passed through Reverse osmosis unit was provided in plastic water bottles with stainless steel sipper tubes. (Except during restraining and exposure period)

The contaminant analysis test reports for the water and bedding material nearest to the experimental period are presented as Annexures 3 and 4 respectively.

6.5 Acclimatization

Healthy young adult animals were acclimatized for six and seven days to laboratory conditions prior to treatment and were observed for clinical signs once daily. On acclimatization day 2 and 3, all the animals were restrained for one hour in the restraining tubes and observed for one hour (±10 minutes) during and post restraining. Pre restraining and post restraining rectal temperature was measured for all the animals. The veterinary examination of all the animals was performed on the day of receipt of animals.

6.6 Study Design

6.6.1 Technical Pre-Test

A technical pre-test without animals was conducted to assess and establish the feasibility of achieving the following objectives:

1. The recommended test atmosphere and stability.
2. Target concentration (5 mg/L of air) or maximum attainable concentration for limit test.
3. Target particle size (MMAD of 1 to 4 µm) with a geometric standard deviation (1.5 to 3.0).

During technical pre-test, the undiluted test item was used to generate the liquid aerosols through collision nebulizer. The technical pre-tests were carried out without animals. Technical pre-tests were conducted by changing different air flow rate between aerosol generator air and dilution air. During the technical pre-test of G1, the mean breathing zone concentration achieved at the air flow rate of 12 L/min with 7.7 L/min aerosol generator air and 4.3 L/min dilution air was 5.04 mg/L of air and the MMAD and GSD obtained were 3.04 µm and 2.62 respectively which were in the prescribed range. During the technical pre-test of G2, the mean breathing zone concentration achieved at the air flow rate of 12 L/min with 7.0 L/min aerosol generator air and 5.0 L/min dilution air was 5.02 mg/L of air and the MMAD and GSD obtained were 3.00 µm and 2.55 respectively which were in the prescribed range. Hence, the achieved concentration for technical pre-test of G1 i.e. 5.04 mg/L of air at the flow rate of 12 L/min with 7.7 L/min aerosol generator air and 4.3 L/min dilution air was considered as the maximum achievable concentration and the achieved concentration for technical pre-test of G2 i.e. 5.02 mg/L of air at the flow rate of 12 L/min with 7.0 L/min aerosol generator air and 5.0 L/min dilution air was considered as the maximum achievable concentration. Therefore, to achieve the maximum concentration in the limit test the air flow rate for technical pre-test of G1 selected was 12 L/min with 7.7 L/min aerosol generator air and 4.3 L/min dilution air and technical pre-test of G2 selected was 12 L/min with 7.0 L/min aerosol generator air and 5.0 L/min dilution air. The mean values obtained for temperature, relative humidity, oxygen and carbon dioxide concentration inside the exposure chamber of technical pre-test of G1 were 22.50°C, 56.30%, 20.35% and 615.50 ppm (0.06%) respectively at the air flow rate of 12 L/min with 7.7 L/min aerosol generator air and 4.3 L/min dilution air and for technical pre-test of G2 were 22.75°C, 56.05%, 20.45% and 617.00 ppm (0.06%) respectively at the air flow rate of 12 L/min with 7.0 L/min aerosol generator air and 5.0 L/min dilution air .

6.6.2 Limit Test

The sighting study was performed with 6 males and 6 females. No mortality was observed at mean maximum achievable concentration of 5.03 mg/L and 5.04 mg/L of air for G1 and G2 respectively. Hence, the sighting study was considered as limit test and no further exposure was conducted.

6.7 Treatment

6.7.1 Route of Administration

The test item was administered through inhalation route by flow-past, nose-only dynamic inhalation exposure unit.

6.7.2 Justification for Selection of Route

Inhalation is one of the possible route of exposure to human.

6.7.3 Duration of Exposure

The animals were exposed to aerosolized test item continuously for four hours after five minutes equilibration period of the chamber concentration.

6.8 Inhalation Exposure System

Inhalation exposure was conducted using a flow-past, nose-only dynamic inhalation exposure system (Refer Annexure 5) supplied by CH Technologies, USA, with minimum of 12 air changes per hour. The exposure unit consisted of stackable exposure tiers with top and bottom sections or plates for introduction and exhaust of test item. Each tier with 12 exposure ports was used to expose 6 animals and another 2 ports were used for gravimetric sampling and chamber atmosphere measurement. All parts (except O-ring seals) were constructed of stainless steel. The volume of each inner plenum of inhalation chamber was 0.76 liters (11 cm diameter and 8 cm height) that consisted of total 12 port holes (one tier). Each tier was made of an inner plenum and an outer plenum that were connected to each other through rectangular trumpets (tubes) and connector cones. The chamber was equipped with a bottom inlet section (plate) and a top exhaust section. Both sections were designed to connect easily to other equipment through swivels. The animals were restrained in the transparent polycarbonate restraining tubes. The chamber was made to sit on a rotating table that allows easy access to observe all animals at each port during exposure. This unit ensured that uniform distribution of test item provided continuous “fresh” test item to each animal and precluded re-breathing the exhaled air. The chamber provided continuous supply of fresh aerosol to all the restrained animals through the trumpet from inner plenum of chamber. A slight positive pressure was maintained in inner plenum of chamber. The animals were confined separately in restraint tubes which were positioned radially around the flow-past, nose-only dynamic inhalation exposure chamber. The whole inhalation chamber was situated inside the fume hood. The outlet air from chamber exhaust was treated with 1% w/v sodium hydroxide solution and passed through absorbent cotton before being evacuated into the atmosphere.

6.9 Test Aerosol Generation

The undiluted liquid test item was aerosolized by using 6 jet collision nebulizer provided by CH Technologies, USA. The test item was filled in to the glass jar and the T Stem shaped tip of the nozzle was immersed in to the test item. The aerosol generator air tube was fitted to air inlet of collision nebulizer and nebulizer outlet fitted to bottom of inhalation chamber. The air was passed at high velocity through the nebulizer's small orifice, it in turn, sucked test item from the nebulizer's jar and breaks it apart into small droplets. The desired air flow was set to generate the target aerosol concentration by using control panel. The generated liquid aerosols were discharged from the top of exposure chamber.

6.10 Exposure System Monitoring

The chamber temperature, relative humidity, oxygen and carbon dioxide concentration were measured during exposure at an empty port of the exposure chamber. The air flow meter (rotameter) was used to regulate and measure, inlet and outlet air of the chamber.

6.10.1 Determination of Nominal Concentration

The nominal concentration was calculated according to "mass of test item disseminated into the exposure system during the generation period divided by the total airflow through the inhalation chamber during the same time period". It was expressed as below:

Group	Pre Weight of Test Item (a)	Post Weight of Test Item (b)	Test Item Utilized (c = a-b)	Air flow rate (L/min) (d)	Duration of Exposure (min) (e)	Nominal Concentration (mg/L) $(e) = \frac{(c)}{(d) \times (e)}$
Sighting Study						
G1	199.7321 g	165.4949 g	34.2372 (34237.2 mg)	12	240	11.89
G2	199.0521 g	166.5838 g	32.4683 g (32468.3 mg)	12	240	11.27

6.10.2 Determination of Breathing Zone Concentration (Actual Concentration)

Breathing zone concentration (actual aerosol concentration) was determined gravimetrically using a 47 mm whatmann filter paper loaded in an in-line sampling device (supplied by CH Technologies, USA). Actual aerosol concentration was determined by gravimetric method i.e. dividing the mass of test item collected on the filter paper by the volume of air passed through the filter paper and time. The 0.84 L/min critical orifice was used to draw the air from the inhalation chamber for one minute at animal breathing zone 3 times during exposure [i.e. 60 minutes (±15 minutes), 120 minutes (±15 minutes) and 180 minutes (±15 minutes)] after equilibration period. The mean breathing zone concentration (actual concentration) for G1 and G2 were 5.03 mg/L and 5.04 mg/L of air respectively during limit test.

6.10.3 Determination of Particle Size Distribution

Particle size distribution was determined gravimetrically by using a 7 stage Cascade Mercer Impactor supplied by In-Tox Products, USA. Based on the results of mass deposited on every stage, Mass Median Aerodynamic Diameters (MMAD) and Geometric Standard Deviations (GSD) were calculated using Microsoft Excel Sheet. The target range for the Mass Median Aerodynamic Diameter was 1 to 4 µm and GSD was 1.5 to 3.0. During sampling, the Impactor air flow rate was 0.84 L/min for one minute. The particle size was measured three times during exposure period at animal breathing zone after equilibration period (i.e. ± 15 minutes from breathing zone concentration sampling).

The cumulative mass less than the stated cut of diameter versus particle size (effective cut of diameter) on log probability scale was plotted to determine MMAD and GSD. During limit test, the mean MMAD and GSD for G1 were found to be 3.02 µm and 2.61 respectively and for G2 were found to be 3.04 µm and 2.57 respectively.

6.10.4 Relative Humidity, Temperature, Oxygen and Carbon Dioxide Concentration

The IAQ probe (Indoor Air Quality probe) supplied by GrayWolf Sensing Solutions, Ireland, was used for continuous monitoring of Relative humidity, temperature, oxygen and carbon dioxide content in the chamber and recorded thrice during exposure (i.e. ± 20 minutes from breathing zone concentration sampling) after equilibration period.

Relative humidity, temperature, oxygen and carbon dioxide content were maintained in the target range of 30% to 70%, 22±3°C, at least 19% and less than 1% respectively. The mean relative humidity, temperature, oxygen and carbon dioxide content for G1 were 56.07%, 22.63°C, 20.37% and 617.67 ppm respectively and the mean relative humidity, temperature, oxygen and carbon dioxide content for G2 were 55.73%, 22.67°C, 20.27% and 619.33 ppm respectively during limit test.

6.10.5 Exposure Air Flow Rate

Air flow through the generation system into the chamber was controlled and monitored through control panel (supplied by CH Technologies, USA) using flow-meters (rotameters) and outlet of the chamber air flow was controlled by rotameter. The actual flow rate of chamber inlet was 12 L/min with 20 psi pressure and outlet air from chamber was 10 L/min maintained throughout the exposure period. The air inlet flow rate was recorded thrice during exposure (i.e. ± 20 minutes from breathing zone concentration sampling) after equilibration period.

6.10.6 Treatment of Exhaust Air

Exhaust air was treated with 1% w/v sodium hydroxide solution (manufactured by: Sd fine chem limited Batch no.:L18A/1018/1711/08, Manufactured date: 12/2018 and Expiry date: 11/2023) and passed through absorbent cotton before evacuated into the atmosphere.

6.11 Observations

The following observations were made during the experiment.

6.11.1 Clinical Signs and Mortality

All the animals were observed for clinical signs and pre-terminal deaths at 1 hr (± 10 mins), 2 hrs (± 10 mins), 3 hrs (± 10 mins) and 4 hrs (± 10 mins) during exposure period. Post exposure, clinical signs and pre-terminal deaths were observed at 30 to 40 minutes and 1 hour (± 10 mins), and once daily thereafter for clinical signs and twice daily for mortality during the 14 days post exposure period. The clinical signs observations included but not limited to changes in skin, fur, eyes, mucous membrane, occurrence of secretions and excretions and autonomic activity such as lacrimation, piloerection, pupil size and unusual respiratory pattern.

6.11.2 Body Weight

Individual animal body weight was recorded at receipt, prior to exposure (day 1) and on day 2, 4, 8 and 15 during the experimental period.

6.11.3 Pathology

6.11.3.1 Necropsy

At the end of the observation period, all the animals were euthanized by intraperitoneal administration of sodium thiopentone and subjected to necropsy and complete gross pathological examination.

Thiopentone details:

Batch No.	:	172432
Manufactured Date	:	07/2020
Expiry Date	:	06/2022
Manufactured by	:	Neon Laboratories Limited

6.11.3.2 Histopathology

Histopathological examination was not carried out as there were no gross lesions observed.

7. STATISTICAL ANALYSIS

Since the study was conducted as a limit test, no statistical analysis was performed for LC_{50}. Microsoft Excel Sheet was used to determine the particle size (MMAD and GSD).

8. CLASSIFICATION

The test item was classified according to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) (Refer Annexure 5).

9. AMENDMENTS AND DEVIATIONS

An amendment was raised to correct the name of Monitoring Scientist as per revised Test Item Data Sheet received from the sponsor and no deviation was occurred during conduct of the study.

10. STUDY REPORT DISTRIBUTION

Original: 1/2 - Sponsor

Original: 2/2 - Archives, Bioneeds India Private Limited.

11. ARCHIVING

All materials and data generated from the study will be stored in the archives of the test facility. The study plan, raw data and study report will be maintained in the archives of Bioneeds India Private Limited for 9 years from the date of completion of the study. At the end of archiving period, the sponsor's instructions will be sought to either extend the archiving period or to return the archived material to the sponsor or for the disposal.

12. REFERENCES

- OECD Guidance Document No. 39 on Acute Inhalation Toxicity Testing; adopted on 06 July 2018.
- Cannon, W. C., Blanton, E. F. and Mc Donald, K. E. (1983). A Flow-Past Chamber: An Improved Nose-Only Exposure System for Rodents. American Industrial Hygiene Association. 44 (12): 923-928.
- The Globally Harmonized System of Classification and Labelling of Chemicals (GHS), 8th edition, 2019 (ST/SG/AC.10/30/REV-8).

13. RESULTS AND DISCUSSION

13.1 Clinical Signs and Mortality

No treatment related clinical signs and mortality were observed at the mean maximum achievable concentration of 5.03 mg/L of air and 5.04 mg/L of air for G1 and G2 respectively.

Refer Table 1

13.2 Body Weight

No treatment related changes were observed in body weight and percent change in body weight with respect to day 1 at the mean maximum achievable concentration of 5.03 mg/L and 5.04 mg/L of air for G1 and G2 respectively. However, all animals showed slight decrease in body weight on day 2 due to exposure and increased in body weight from day 4 onwards.

Refer Table 2

13.3 Chamber (Exposure) Conditions

During the limit test, the temperature, relative humidity, oxygen and carbon dioxide concentration of the chamber were 22.4°C to 22.8°C, 55.8% to 56.3%, 20.2% to 20.5% and 616 ppm to 619 ppm (0.06%) respectively for G1. The particle size MMAD and GSD were 2.90 µm to 3.13 µm and 2.61 to 2.62 respectively. All the values were within the range. The mean maximum achievable breathing zone concentration (actual concentration) was 5.03 mg/L of air and it was considered as the limit concentration.

During the limit test, the temperature, relative humidity, oxygen and carbon dioxide concentration of the chamber were 22.4°C to 22.9°C, 55.4% to 56.2%, 20.1% to 20.4% and 619 ppm to 620 ppm (0.06%) respectively for G2. The particle size MMAD and GSD were 2.91 µm to 3.12 µm and 2.56 to 2.58 respectively. All the values were within the range. The mean maximum achievable breathing zone concentration (actual concentration) was 5.04 mg/L of air and it was considered as the limit concentration.

Refer Tables 3, 4, 6 and 7

13.4 Pathology

No treatment related gross pathological findings were observed at the maximum achievable concentration of 5.03 mg/L of air and 5.04 mg/L of air for G1 and G2 during limit test respectively.

Refer Table 8

14. CONCLUSION

Under the experimental conditions employed and based on the above results of experiment, there were no clinical signs and mortality observed at mean maximum achievable concentration of 5.03 mg/L for G1 and 5.04 mg/L of air for G2. Hence, the LC_{50} of the test item, BIO-X Kleanze EC is >5.03 and 5.04 mg/L of air and is classified as “Category 5” according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

15. TABLES

TABLE 1. CLINICAL SIGNS AND MORTALITY RECORD

Group, Phase & Concentration (mg/L of air)	Animal No.	Sex	Day 1						Days													
			During Exposure				Post exposure															
			1hr*	2hrs*	3hrs*	4hrs*	30-40 min	1hr*	2	3	4	5	6	7	8	9	10	11	12	13	14	15
G1, Limit Test & 5.03	Rf0195	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Rf0196	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Rf0197	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Rf0198	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Rf0199	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Rf0200	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

*: ± 10 minutes; N: Normal; M: Male; F: Female; min: minute; hr(s): hour(s)

TABLE 1 (Contd…). CLINICAL SIGNS AND MORTALITY RECORD

Group, Phase & Concentration (mg/L of air)	Animal No.	Sex	Day 1						Days													
			During Exposure				Post exposure															
			1hr*	2hrs*	3hrs*	4hrs*	30-40 min	1hr*	2	3	4	5	6	7	8	9	10	11	12	13	14	15
G2, Limit Test & 5.04	Rf0201	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Rf0202	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Rf0203	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Rf0204	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Rf0205	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Rf0206	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

*: ± 10 minutes; N: Normal; M: Male; F: Female; min: minute; hr(s): hour(s)

TABLE 2. BODY WEIGHT (g) AND PERCENT CHANGE IN BODY WEIGHT WITH RESPECT TO DAY 1

Group, Phase & Concentration (mg/L of air)	Animal No.	Sex	Body Weight (g) on Days					Percent Change in Body Weight with Respect to Day 1			
			1#	2	4	8	15	1-2	1-4	1-8	1-15
G1, Limit Test & 5.03	Rf0195	M	206.24	204.43	207.65	222.44	246.14	-0.88	0.68	7.85	19.35
	Rf0196	M	212.19	210.69	214.40	228.68	253.07	-0.71	1.04	7.77	19.27
	Rf0197	M	205.87	204.12	207.93	221.12	247.22	-0.85	1.00	7.41	20.09
	Mean		**208.10**	**206.41**	**209.99**	**224.08**	**248.81**	**-0.81**	**0.91**	**7.68**	**19.57**
	(±) SD		**3.55**	**3.71**	**3.82**	**4.04**	**3.73**	**0.09**	**0.20**	**0.24**	**0.45**
	Rf0198	F	178.12	177.74	180.22	189.09	205.60	-0.21	1.18	6.16	15.43
	Rf0199	F	187.09	186.16	189.69	199.56	216.47	-0.50	1.39	6.67	15.70
	Rf0200	F	183.55	184.09	186.14	196.30	213.79	0.29	1.41	6.95	16.48
	Mean		**182.92**	**182.66**	**185.35**	**194.98**	**211.95**	**-0.14**	**1.33**	**6.59**	**15.87**
	(±) SD		**4.52**	**4.39**	**4.78**	**5.36**	**5.66**	**0.40**	**0.13**	**0.40**	**0.54**

#: Prior to exposure; M: Male; F: Female; SD: Standard Deviation

TABLE 2 (Contd…). BODY WEIGHT (g) AND PERCENT CHANGE IN BODY WEIGHT WITH RESPECT TO DAY 1

Group, Phase & Concentration (mg/L of air)	Animal No.	Sex	Body Weight (g) on Days					Percent Change in Body Weight with Respect to Day 1			
			1#	2	4	8	15	1-2	1-4	1-8	1-15
G2, Limit Test & 5.04	**Rf0201**	**M**	211.22	209.12	213.02	226.22	251.73	-0.99	0.85	7.10	19.18
	Rf0202	**M**	219.14	217.52	220.80	235.59	261.12	-0.74	0.76	7.51	19.16
	Rf0203	**M**	218.69	217.09	220.96	234.16	260.69	-0.73	1.04	7.07	19.21
	Mean		**216.35**	**214.58**	**218.26**	**231.99**	**257.85**	**-0.82**	**0.88**	**7.23**	**19.18**
	(±) SD		**4.45**	**4.73**	**4.54**	**5.05**	**5.30**	**0.15**	**0.14**	**0.24**	**0.02**
	Rf0204	**F**	187.06	186.40	189.21	198.04	214.38	-0.35	1.15	5.87	14.60
	Rf0205	**F**	182.21	180.49	182.59	192.38	209.09	-0.94	0.21	5.58	14.75
	Rf0206	**F**	185.10	184.02	186.35	196.01	213.11	-0.58	0.68	5.89	15.13
	Mean		**184.79**	**183.64**	**186.05**	**195.48**	**212.19**	**-0.63**	**0.68**	**5.78**	**14.83**
	(±) SD		**2.44**	**2.97**	**3.32**	**2.87**	**2.76**	**0.30**	**0.47**	**0.17**	**0.27**

#: Prior to exposure; M: Male; F: Female; SD: Standard Deviation

TABLE 3. BREATHING ZONE CONCENTRATION (ACTUAL TEST ITEM CONCENTRATION)

Technical Pre-test - G1:

Sl. No.	Aerosol generation air (L/min) (a)	Dilution air (L/min) (b)	Total Air flow rate (L/min) (c) = (a) + (b)	Initial weight (mg) (d)	Final weight (mg) (e)	Difference (mg) (f) = (e) – (d)	Air Flow Rate (L/min) (g)	Time (min) (h)	BZC (mg/L of air) $(i) = \frac{(f)}{(g) \times (h)}$	Mean BZC (mg/L of air)
1	7.7	4.3	12	343.09	347.31	4.22	0.84	1	5.02	**5.04**
2	7.7	4.3	12	343.74	347.99	4.25	0.84	1	5.06	

Limit Test - G1:

Sl. No.	Aerosol generation air (L/min) (a)	Dilution air (L/min) (b)	Total Air flow rate (L/min) (c) = (a) + (b)	Initial weight (mg) (d)	Final weight (mg) (e)	Difference (mg) (f) = (e) – (d)	Air Flow Rate (L/min) (g)	Time (min) (h)	BZC (mg/L of air) $(i) = \frac{(f)}{(g) \times (h)}$	Mean BZC (mg/L of air)
1	7.7	4.3	12	345.52	349.75	4.23	0.84	1	5.04	
2	7.7	4.3	12	345.72	349.92	4.20	0.84	1	5.00	**5.03**
3	7.7	4.3	12	344.67	348.92	4.25	0.84	1	5.06	

BZC: Breathing Zone Concentration; Sampled volume: 0.84 L/min; Sampling time: 1 minute

$$\text{BZC (i)} = \frac{\text{Mass of test item collected on the filter paper (f)}}{\text{volume of air passed through the filter paper (g) X time (h)}}$$

TABLE 3 (Contd…). BREATHING ZONE CONCENTRATION (ACTUAL TEST ITEM CONCENTRATION)

Technical Pre-test - G2:

Sl. No.	Aerosol generation air (L/min) (a)	Dilution air (L/min) (b)	Total Air flow rate (L/min) (c) = (a) + (b)	Initial weight (mg) (d)	Final weight (mg) (e)	Difference (mg) (f) = (e) – (d)	Air Flow Rate (L/min) (g)	Time (min) (h)	BZC (mg/L of air) (i) = (f) / (g) × (h)	Mean BZC (mg/L of air)
1	7.0	5.0	12	343.46	347.69	4.23	0.84	1	5.04	**5.02**
2	7.0	5.0	12	344.25	348.46	4.21	0.84	1	5.01	

Limit Test - G2:

Sl. No.	Aerosol generation air (L/min) (a)	Dilution air (L/min) (b)	Total Air flow rate (L/min) (c) = (a) + (b)	Initial weight (mg) (d)	Final weight (mg) (e)	Difference (mg) (f) = (e) – (d)	Air Flow Rate (L/min) (g)	Time (min) (h)	BZC (mg/L of air) (i) = (f) / (g) × (h)	Mean BZC (mg/L of air)
1	7.0	5.0	12	344.81	349.03	4.22	0.84	1	5.02	
2	7.0	5.0	12	345.62	349.87	4.25	0.84	1	5.06	**5.04**
3	7.0	5.0	12	345.15	349.38	4.23	0.84	1	5.04	

BZC: Breathing Zone Concentration; Sampled volume: 0.84 L/min; Sampling time: 1 minute

$$\text{BZC (i)} = \frac{\text{Mass of test item collected on the filter paper (f)}}{\text{volume of air passed through the filter paper (g) X time (h)}}$$

TABLE 4. CHAMBER (EXPOSURE) CONDITIONS

Technical Pre-test - G1:

Concentration (mg/L of air)	SL. No.	Aerosol generation air (L/min) (a)	Dilution air (L/min) (b)	Total Air flow rate (L/min) (c) = (a) + (b)	Temperature (°C)	Relative Humidity (%)	Oxygen Concentration (%)	Carbon dioxide Concentration (ppm)
5.04	1	7.7	4.3	12	22.4	56.5	20.4	615
	2	7.7	4.3	12	22.6	56.1	20.3	616

Limit Test - G1:

Concentration (mg/L of air)	SL. No.	Aerosol generation air (L/min) (a)	Dilution air (L/min) (b)	Total Air flow rate (L/min) (c) = (a) + (b)	Temperature (°C)	Relative Humidity (%)	Oxygen Concentration (%)	Carbon dioxide Concentration (ppm)
5.03	1	7.7	4.3	12	22.7	55.8	20.5	618
	2	7.7	4.3	12	22.4	56.3	20.2	616
	3	7.7	4.3	12	22.8	56.1	20.4	619

Note: 1% Carbon dioxide = 10000 ppm

TABLE 4 (Contd…). CHAMBER (EXPOSURE) CONDITIONS

Technical Pre-test - G2:

Concentration (mg/L of air)	SL. No.	Aerosol generation air (L/min) (a)	Dilution air (L/min) (b)	Total Air flow rate (L/min) (c) = (a) + (b)	Temperature (°C)	Relative Humidity (%)	Oxygen Concentration (%)	Carbon dioxide Concentration (ppm)
5.02	1	7.0	5.0	12	22.6	55.9	20.4	615
	2	7.0	5.0	12	22.9	56.2	20.5	619

Limit Test - G2:

Concentration (mg/L of air)	SL. No.	Aerosol generation air (L/min) (a)	Dilution air (L/min) (b)	Total Air flow rate (L/min) (c) = (a) + (b)	Temperature (°C)	Relative Humidity (%)	Oxygen Concentration (%)	Carbon dioxide Concentration (ppm)
5.04	1	7.0	5.0	12	22.4	55.6	20.3	620
	2	7.0	5.0	12	22.9	55.4	20.1	619
	3	7.0	5.0	12	22.7	56.2	20.4	619

Note: 1% Carbon dioxide = 10000 ppm

TABLE 5. PARTICLE SIZE DISTRIBUTION DURING TECHNICAL PRE-TEST

BIO-EXV-TOX/020 Version No. 01

BIONEEDS

CALCUALATION OF MMAD and GSD

Date : 28-Dec-20

Study No. : **BIO-ITX 259** Test Item code : D1155-001

Determination Method : Gravimetric

Group : Technical-Pre Test G1(without animals)

Size Range (µm)	ECD (µm)	Pre-weight (mg)	Post-weight (mg)	Net weight (mg)	Cumulative mass on Stage	Percentage on Stage	Cumulative Percentage < Stage	Log ECD	Probit
>5.19	--	372.200	372.330	0.130	0.550	23.64	100.00		
3.38 - 5.19	5.190	373.530	373.640	0.110	0.420	20.00	76.36	0.715	5.718
2.34 - 3.38	3.380	377.360	377.460	0.100	0.310	18.18	56.36	0.529	5.160
1.82 -2.34	2.340	374.640	374.720	0.080	0.210	14.55	38.18	0.369	4.699
1.19 -1.82	1.820	378.810	378.870	0.060	0.130	10.91	23.64	0.260	4.282
0.80 - 1.19	1.190	375.220	375.260	0.040	0.070	7.27	12.73	0.076	3.861
0.28 - 0.80	0.800	379.260	379.280	0.020	0.030	3.64	5.45	-0.097	3.398
0.0 - 0.28	0.280	497.150	497.160	0.010	0.010	1.82	1.82	-0.553	2.907
			Sum Net weight:	0.550					

ECD: Effective cut-off diameter

Correlation coefficient (r) :	0.977
Slope :	0.418
y-Axis :	-1.609
Number of data points :	7

Cum. Weight %	Probit value	Log_{10} ECD	ECD
15.9	4.001	0.065	1.16
50.0	5.000	0.483	3.04
84.1	5.999	0.901	7.95

MMAD (µm) :	**3.04**
GSD :	**2.62**

Particle Size Determination Method : Gravimetric Determination
Group : Technical-Pre Test G1(without animals) on 28-Dec-20

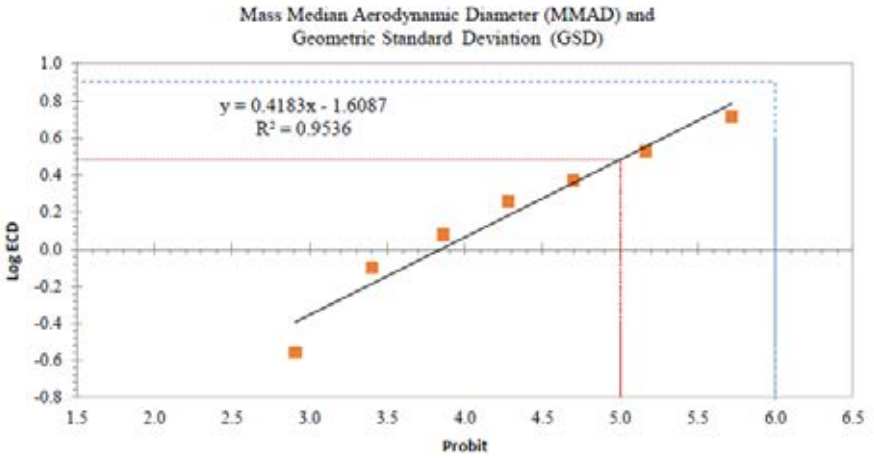

TABLE 5 (Contd…). PARTICLE SIZE DISTRIBUTION DURING TECHNICAL PRE-TEST

BIO-EXV-TOX/020 Version No. 01

BIONEEDS

CALCUALATION OF MMAD and GSD

Date : 29-Dec-20

Study No. : **BIO-ITX 259** Test Item code : D1155-001

Determination Method : Gravimetric

Group : Technical-Pre Test G2(without animals)

Size Range (µm)	ECD (µm)	Pre-weight (mg)	Post-weight (mg)	Net weight (mg)	Cumulative mass on Stage	Percentage on Stage	Cumulative Percentage < Stage	Log ECD	Probit
>5.19	--	376.120	376.260	0.140	0.600	23.33	100.00		
3.38 - 5.19	5.190	379.650	379.770	0.120	0.460	20.00	76.67	0.715	5.728
2.34 - 3.38	3.380	373.970	374.070	0.100	0.340	16.67	56.67	0.529	5.168
1.82 -2.34	2.340	372.150	372.240	0.090	0.240	15.00	40.00	0.369	4.747
1.19 -1.82	1.820	377.200	377.280	0.080	0.150	13.33	25.00	0.260	4.326
0.80 - 1.19	1.190	374.940	374.980	0.040	0.070	6.67	11.67	0.076	3.808
0.28 - 0.80	0.800	378.660	378.680	0.020	0.030	3.33	5.00	-0.097	3.355
0.0 - 0.28	0.280	497.150	497.160	0.010	0.010	1.67	1.67	-0.553	2.872
			Sum Net weight:	0.600					

ECD: Effective cut-off diameter

Correlation coefficient (r) :	0.977
Slope :	0.408
y-Axis :	-1.562
Number of data points :	7

Cum. Weight %	Probit value	Log_{10} ECD	ECD
15.9	4.001	0.069	1.17
50.0	5.000	0.477	3.00
84.1	5.999	0.884	7.65

MMAD (µm) :	**3.00**
GSD :	**2.55**

Particle Size Determination Method : Gravimetric Determination
Group : Technical-Pre Test G2(without animals) on 29-Dec-20

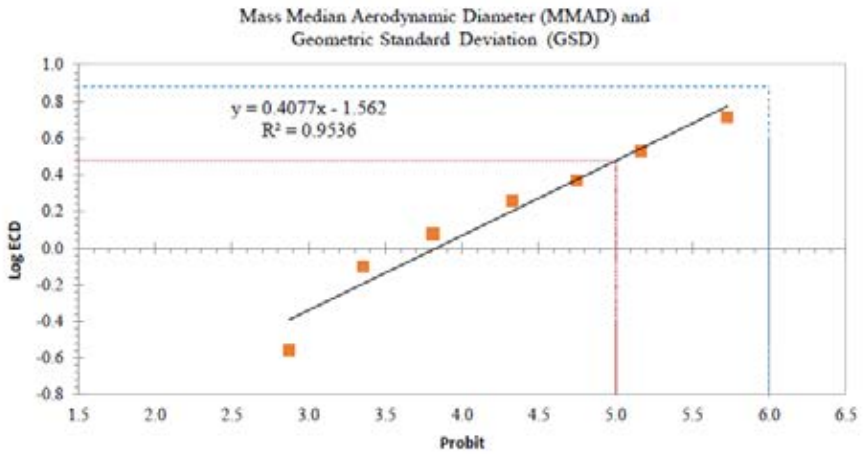

TABLE 6. PARTICLE SIZE DISTRIBUTION DURING LIMIT TEST

BIO-EXV-TOX/020 Version No. 01

BIONEEDS

CALCUALATION OF MMAD and GSD

Date : 28-Dec-20

Study No. : **BIO-ITX 259** Test Item code : D1155-001

Determination Method : Gravimetric

Group : Limit-Test Cascade 1(G1)

Size Range (µm)	ECD (µm)	Pre-weight (mg)	Post-weight (mg)	Net weight (mg)	Cumulative mass on Stage	Percentage on Stage	Cumulative Percentage < Stage	Log ECD	Probit
>5.19	--	372.620	372.760	0.140	0.620	22.58	100.00		
3.38 - 5.19	5.190	373.460	373.580	0.120	0.480	19.35	77.42	0.715	5.753
2.34 - 3.38	3.380	375.210	375.320	0.110	0.360	17.74	58.06	0.529	5.204
1.82 -2.34	2.340	374.200	374.290	0.090	0.250	14.52	40.32	0.369	4.755
1.19 -1.82	1.820	376.130	376.200	0.070	0.160	11.29	25.81	0.260	4.351
0.80 - 1.19	1.190	378.380	378.430	0.050	0.090	8.06	14.52	0.076	3.943
0.28 - 0.80	0.800	379.400	379.430	0.030	0.040	4.84	6.45	-0.097	3.482
0.0 - 0.28	0.280	497.160	497.170	0.010	0.010	1.61	1.61	-0.553	2.859
			Sum Net weight:	0.620					

ECD: Effective cut-off diameter

Correlation coefficient (r) :	0.985
Slope :	0.417
y-Axis :	-1.621
Number of data points :	7

Cum. Weight %	Probit value	Log_{10} ECD	ECD
15.9	4.001	0.047	1.11
50.0	5.000	0.463	2.90
84.1	5.999	0.879	7.57

MMAD (µm) :	**2.90**
GSD :	**2.61**

Particle Size Determination Method : Gravimetric Determination
Group : Limit-Test Cascade 1(G1) on 28-Dec-20

Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD)

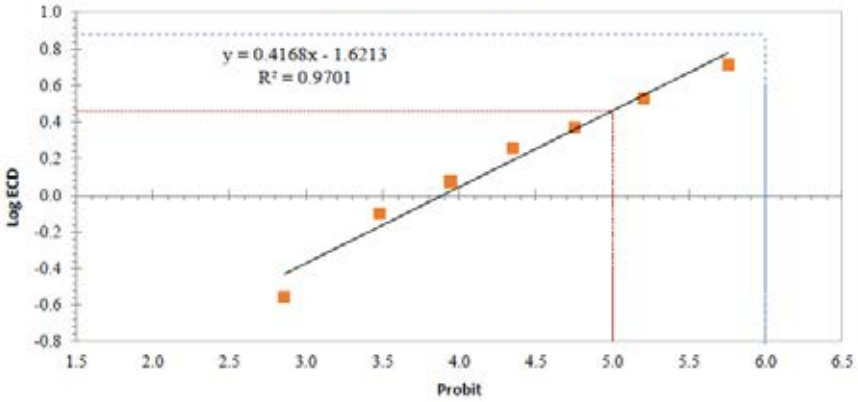

TABLE 6 (Contd…). PARTICLE SIZE DISTRIBUTION DURING LIMIT TEST

BIO-EXV-TOX/020 Version No. 01

BIONEEDS

CALCUALATION OF MMAD and GSD

Date : 28-Dec-20

Study No. : **BIO-ITX 259** Test Item code : D1155-001

Determination Method : Gravimetric

Group : Limit-Test Cascade 2(G1)

Size Range (µm)	ECD (µm)	Pre-weight (mg)	Post-weight (mg)	Net weight (mg)	Cumulative mass on Stage	Percentage on Stage	Cumulative Percentage < Stage	Log ECD	Probit
>5.19	--	373.420	373.550	0.130	0.550	23.64	100.00		
3.38 - 5.19	5.190	379.430	379.540	0.110	0.420	20.00	76.36	0.715	5.718
2.34 - 3.38	3.380	378.360	378.460	0.100	0.310	18.18	56.36	0.529	5.160
1.82 -2.34	2.340	376.170	376.250	0.080	0.210	14.55	38.18	0.369	4.699
1.19 -1.82	1.820	375.250	375.310	0.060	0.130	10.91	23.64	0.260	4.282
0.80 - 1.19	1.190	374.200	374.240	0.040	0.070	7.27	12.73	0.076	3.861
0.28 - 0.80	0.800	372.660	372.680	0.020	0.030	3.64	5.45	-0.097	3.398
0.0 - 0.28	0.280	497.140	497.150	0.010	0.010	1.82	1.82	-0.553	2.907
			Sum Net weight:	0.550					

ECD: Effective cut-off diameter

Correlation coefficient (r) :	0.977
Slope :	0.418
y-Axis :	-1.609
Number of data points :	7

Cum. Weight %	Probit value	Log_{10} ECD	ECD
15.9	4.001	0.065	1.16
50.0	5.000	0.483	3.04
84.1	5.999	0.901	7.95

MMAD (µm) :	**3.04**
GSD :	**2.62**

Particle Size Determination Method : Gravimetric Determination

Group : Limit-Test Cascade 2(G1) on 28-Dec-20

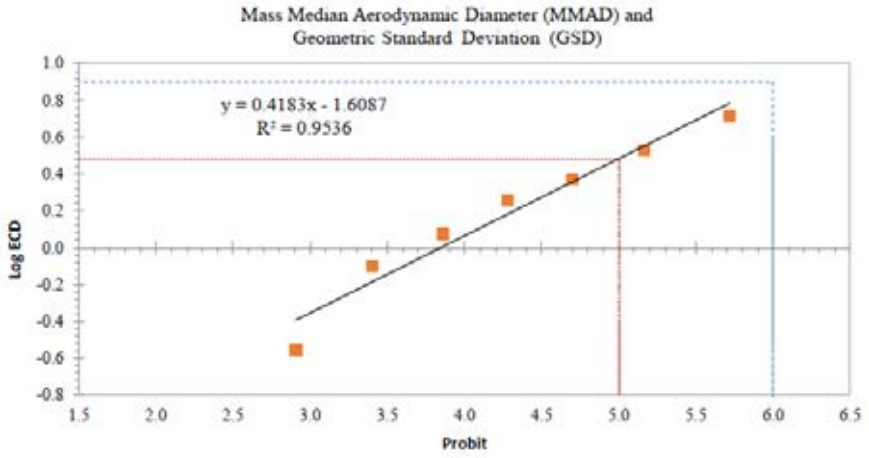

TABLE 6 (Contd…). PARTICLE SIZE DISTRIBUTION DURING LIMIT TEST

BIO-EXV-TOX/020 Version No. 01

BIONEEDS

CALCUALATION OF MMAD and GSD

Date : 28-Dec-20

Study No. : **BIO-ITX 259** Test Item code : D1155-001

Determination Method : Gravimetric

Group : Limit-Test Cascade 3(G1)

Size Range (µm)	ECD (µm)	Pre-weight (mg)	Post-weight (mg)	Net weight (mg)	Cumulative mass on Stage	Percentage on Stage	Cumulative Percentage < Stage	Log ECD	Probit
>5.19	--	374.310	374.450	0.140	0.580	24.14	100.00		
3.38 - 5.19	5.190	376.240	376.360	0.120	0.440	20.69	75.86	0.715	5.702
2.34 - 3.38	3.380	375.400	375.510	0.110	0.320	18.97	55.17	0.529	5.130
1.82 -2.34	2.340	378.230	378.310	0.080	0.210	13.79	36.21	0.369	4.647
1.19 -1.82	1.820	379.500	379.560	0.060	0.130	10.34	22.41	0.260	4.242
0.80 - 1.19	1.190	375.240	375.280	0.040	0.070	6.90	12.07	0.076	3.828
0.28 - 0.80	0.800	379.380	379.400	0.020	0.030	3.45	5.17	-0.097	3.372
0.0 - 0.28	0.280	497.160	497.170	0.010	0.010	1.72	1.72	-0.553	2.886
			Sum Net weight:	0.580					

ECD: Effective cut-off diameter

Correlation coefficient (r) :	0.975
Slope :	0.418
y-Axis :	-1.594
Number of data points :	7

Cum. Weight %	Probit value	Log_{10} ECD	ECD
15.9	4.001	0.078	1.20
50.0	5.000	0.496	3.13
84.1	5.999	0.913	8.19

MMAD (µm) :	**3.13**
GSD :	**2.61**

Particle Size Determination Method : Gravimetric Determination
Group : Limit-Test Cascade 3(G1) on 28-Dec-20

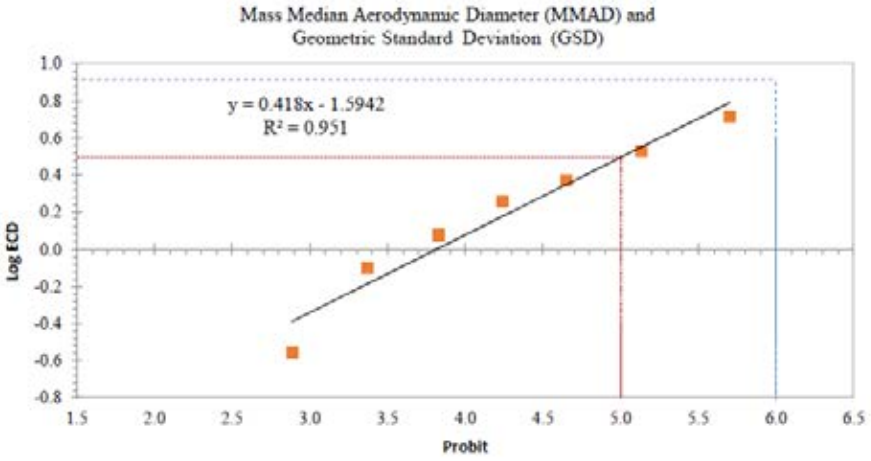

TABLE 6 (Contd…). PARTICLE SIZE DISTRIBUTION DURING LIMIT TEST

BIO-EXV-TOX/020 Version No. 01

BIONEEDS

CALCUALATION OF MMAD and GSD

Date : 29-Dec-20

Study No. : **BIO-ITX 259** Test Item code : D1155-001

Determination Method : Gravimetric

Group : Limit-Test Cascade 1(G2)

Size Range (µm)	ECD (µm)	Pre-weight (mg)	Post-weight (mg)	Net weight (mg)	Cumulative mass on Stage	Percentage on Stage	Cumulative Percentage < Stage	Log ECD	Probit
>5.19	--	379.220	379.370	0.150	0.630	23.81	100.00		
3.38 - 5.19	5.190	377.100	377.230	0.130	0.480	20.63	76.19	0.715	5.712
2.34 - 3.38	3.380	372.570	372.690	0.120	0.350	19.05	55.56	0.529	5.140
1.82 -2.34	2.340	375.160	375.250	0.090	0.230	14.29	36.51	0.369	4.655
1.19 -1.82	1.820	373.060	373.130	0.070	0.140	11.11	22.22	0.260	4.235
0.80 - 1.19	1.190	377.800	377.840	0.040	0.070	6.35	11.11	0.076	3.779
0.28 - 0.80	0.800	374.670	374.690	0.020	0.030	3.17	4.76	-0.097	3.332
0.0 - 0.28	0.280	497.140	497.150	0.010	0.010	1.59	1.59	-0.553	2.852
			Sum Net weight:	0.630					

ECD: Effective cut-off diameter

Correlation coefficient (r) :	0.974
Slope :	0.408
y-Axis :	-1.548
Number of data points :	7

Cum. Weight %	Probit value	Log_{10} ECD	ECD
15.9	4.001	0.087	1.22
50.0	5.000	0.494	3.12
84.1	5.999	0.902	7.99

MMAD (µm) :	**3.12**
GSD :	**2.56**

Particle Size Determination Method : Gravimetric Determination

Group : Limit-Test Cascade 1(G2) on 29-Dec-20

Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD)

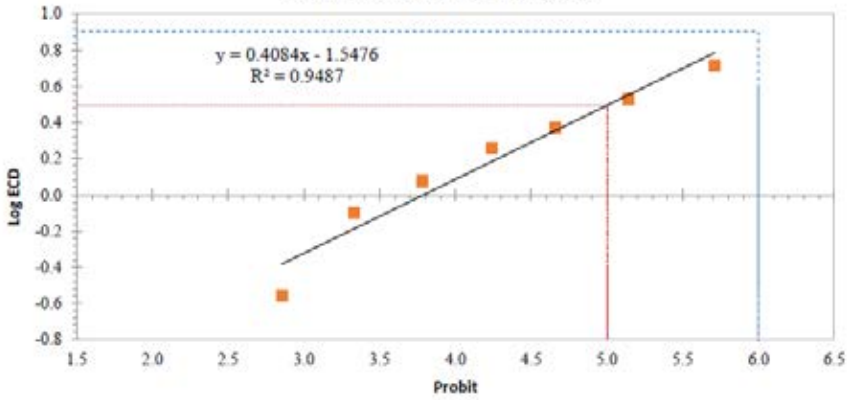

TABLE 6 (Contd…). PARTICLE SIZE DISTRIBUTION DURING LIMIT TEST

BIO-EXV-TOX/020 Version No. 01

BIONEEDS

CALCUALATION OF MMAD and GSD

Date : 29-Dec-20

Study No. : **BIO-ITX 259** Test Item code : D1155-001

Determination Method : Gravimetric

Group : Limit-Test Cascade 2(G2)

Size Range (µm)	ECD (µm)	Pre-weight (mg)	Post-weight (mg)	Net weight (mg)	Cumulative mass on Stage	Percentage on Stage	Cumulative Percentage < Stage	Log ECD	Probit
>5.19	--	375.260	375.400	0.140	0.590	23.73	100.00		
3.38 - 5.19	5.190	372.120	372.240	0.120	0.450	20.34	76.27	0.715	5.715
2.34 - 3.38	3.380	379.440	379.550	0.110	0.330	18.64	55.93	0.529	5.149
1.82 -2.34	2.340	373.930	374.020	0.090	0.220	15.25	37.29	0.369	4.676
1.19 -1.82	1.820	374.160	374.220	0.060	0.130	10.17	22.03	0.260	4.229
0.80 - 1.19	1.190	377.010	377.050	0.040	0.070	6.78	11.86	0.076	3.818
0.28 - 0.80	0.800	378.590	378.610	0.020	0.030	3.39	5.08	-0.097	3.363
0.0 - 0.28	0.280	497.130	497.140	0.010	0.010	1.69	1.69	-0.553	2.879
			Sum Net weight:	0.590					

ECD: Effective cut-off diameter

Correlation coefficient (r) :	0.974
Slope :	0.413
y-Axis :	-1.573
Number of data points :	7

Cum. Weight %	Probit value	Log_{10} ECD	ECD
15.9	4.001	0.078	1.20
50.0	5.000	0.490	3.09
84.1	5.999	0.903	7.99

MMAD (µm) :	**3.09**
GSD :	**2.58**

Particle Size Determination Method : Gravimetric Determination

Group : Limit-Test Cascade 2(G2) on 29-Dec-20

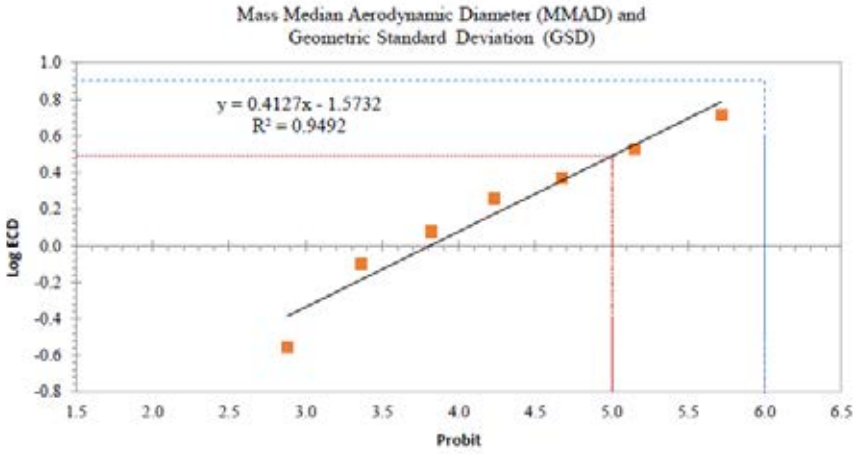

TABLE 6 (Contd…). PARTICLE SIZE DISTRIBUTION DURING LIMIT TEST

BIO-EXV-TOX/020 Version No. 01

BIONEEDS

CALCUALATION OF MMAD and GSD

Date : 29-Dec-20

Study No. : **BIO-ITX 259** Test Item code : D1155-001

Determination Method : Gravimetric

Group : Limit-Test Cascade 3(G2)

Size Range (µm)	ECD (µm)	Pre-weight (mg)	Post-weight (mg)	Net weight (mg)	Cumulative mass on Stage	Percentage on Stage	Cumulative Percentage < Stage	Log ECD	Probit
>5.19	--	372.400	372.550	0.150	0.670	22.39	100.00		
3.38 - 5.19	5.190	375.280	375.410	0.130	0.520	19.40	77.61	0.715	5.759
2.34 - 3.38	3.380	373.800	373.920	0.120	0.390	17.91	58.21	0.529	5.207
1.82 -2.34	2.340	379.460	379.560	0.100	0.270	14.93	40.30	0.369	4.754
1.19 -1.82	1.820	377.090	377.170	0.080	0.170	11.94	25.37	0.260	4.337
0.80 - 1.19	1.190	374.200	374.250	0.050	0.090	7.46	13.43	0.076	3.894
0.28 - 0.80	0.800	378.550	378.580	0.030	0.040	4.48	5.97	-0.097	3.443
0.0 - 0.28	0.280	497.140	497.150	0.010	0.010	1.49	1.49	-0.553	2.828
			Sum Net weight:	0.670					

ECD: Effective cut-off diameter

Correlation coefficient (r) :	0.984
Slope :	0.409
y-Axis :	-1.579
Number of data points :	7

Cum. Weight %	Probit value	Log_{10} ECD	ECD
15.9	4.001	0.056	1.14
50.0	5.000	0.465	2.91
84.1	5.999	0.873	7.46

MMAD (µm) :	**2.91**
GSD :	**2.56**

Particle Size Determination Method : Gravimetric Determination

Group : Limit-Test Cascade 3(G2) on 29-Dec-20

Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD)

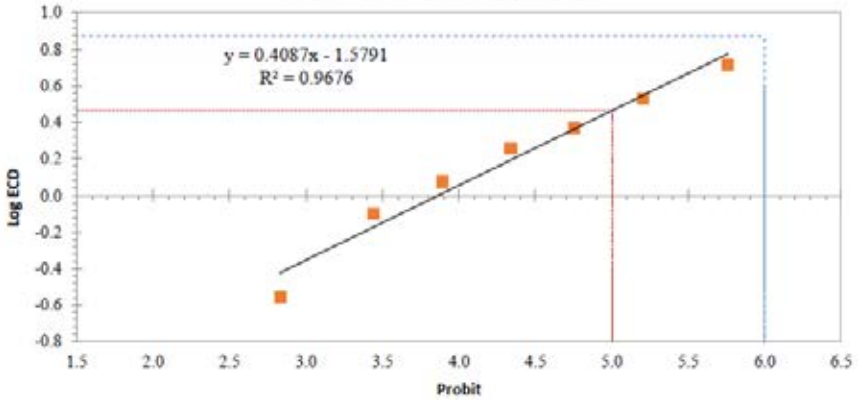

TABLE 7. SUMMARY OF CHAMBER (EXPOSURE) CONDITIONS

Technical Pre Test G1	
Chamber Conditions	**Range**
Temperature (°C)	22.4 to 22.6
Relative humidity (%)	56.1 to 56.5
Oxygen concentration (%)	20.3 to 20.4
Carbon dioxide concentration (ppm)	615 to 616
Air inlet (L/min)*	12 (7.7 L/min aerosol generator air + 4.3 L/min dilution air)
BZC (mg/L)	5.02 to 5.06
MMAD (µm)	3.04 #
GSD	2.62 #
Limit Test G1	
Temperature (°C)	22.4 to 22.8
Relative humidity (%)	55.8 to 56.3
Oxygen concentration (%)	20.2 to 20.5
Carbon dioxide concentration (ppm)	616 to 619
Air inlet (L/min)*	12 (7.7 L/min aerosol generator air + 4.3 L/min dilution air)
BZC (mg/L)	5.00 to 5.06
MMAD (µm)	2.90 to 3.13
GSD	2.61 to 2.62

*: Values were constant throughout the exposure; #: Individual value; BZC: Breathing Zone Concentration (Actual Concentration); MMAD: Mass Median Aerodynamic Diameter; GSD: Geometric Standard Deviation.

TABLE 7 (Contd…). SUMMARY OF CHAMBER (EXPOSURE) CONDITIONS

Technical Pre Test G2	
Chamber Conditions	**Range**
Temperature (°C)	22.6 to 22.9
Relative humidity (%)	55.9 to 56.2
Oxygen concentration (%)	20.4 to 20.5
Carbon dioxide concentration (ppm)	615 to 619
Air inlet (L/min)*	12 (7.0 L/min aerosol generator air + 5.0 L/min dilution air)
BZC (mg/L)	5.01 to 5.04
MMAD (µm)	3.00 #
GSD	2.55 #
Limit Test G2	
Temperature (°C)	22.4 to 22.9
Relative humidity (%)	55.4 to 56.2
Oxygen concentration (%)	20.1 to 20.4
Carbon dioxide concentration (ppm)	619 to 620
Air inlet (L/min)*	12 (7.0 L/min aerosol generator air + 5.0 L/min dilution air)
BZC (mg/L)	5.02 to 5.06
MMAD (µm)	2.91 to 3.12
GSD	2.56 to 2.58

*: Values were constant throughout the exposure; #: Individual value; BZC: Breathing Zone Concentration (Actual Concentration); MMAD: Mass Median Aerodynamic Diameter; GSD: Geometric Standard Deviation.

TABLE 8. GROSS PATHOLOGY FINDINGS

Group, Phase & Concentration (mg/L of air)	Animal No.	Sex	Fate	Gross Pathology Findings	
				External	Internal
G1 Limit Test & 5.03	Rf0195	M	TS	NAD	NAD
	Rf0196	M	TS	NAD	NAD
	Rf0197	M	TS	NAD	NAD
	Rf0198	F	TS	NAD	NAD
	Rf0199	F	TS	NAD	NAD
	Rf0200	F	TS	NAD	NAD
G2 Limit Test & 5.04	Rf0201	M	TS	NAD	NAD
	Rf0202	M	TS	NAD	NAD
	Rf0203	M	TS	NAD	NAD
	Rf0204	F	TS	NAD	NAD
	Rf0205	F	TS	NAD	NAD
	Rf0206	F	TS	NAD	NAD

NAD: No Abnormality Detected; M: Male; F: Female; TS: Terminal Sacrifice

16. ANNEXURES

ANNEXURE 1. CERTIFICATE OF ANALYSIS OF BIO-X KLEANZE EC

OKADA ECOTECH PTE LTD (REG NO 199805564E)
24 Pioneer Crescent, #04-08, West Park Bizcentral, Singapore 628557
Tel: (65) 6872 3515 Fax: (65) 6872 6558
Website: www.okada-ecotech.com

CERTIFICATE OF ANALYSIS

Attention : **To whom it may concern**

Product Name : **Bio-X® Kleanze EC**

Batch Number : 2020061201

Date of Test : 1 December 2020

TEST	SPECIFICATIONS	RESULT
Appearance	Clear brown	OK
Odor	Pleasant	OK
Viscosity (cP) (BF DVII #1/100RPM/30Deg C)	20 ± 5 cps	20.0
Specific Gravity	0.95 ± 0.10	0.96
Dispersibility in Water	All proportion dispersible	OK

Tan Aik Zen
(Chemical Engineer)

ANNEXURE 2. CONTAMINANT ANALYSIS TEST REPORT OF FEED

Nr.: QA - 72
Aufbewahrungsdauer:
15 Jahre nach Erstellen

Altromin Spezialfutter GmbH & Co. KG
Im Seelenkamp 20
D-32791 Lage
Tel. +49 (0)5232 / 6088-0
Fax. +49 (0)5232 / 6088-20
E-Mail: analysen@altromin.de

altromin

Producer Certificate

Description	Maintenance diet for rats and mice
Type	1324
	Isoflavone Genistein <350 ppm
	10mm pellets, 12.5 kg double plastic bags
Customer	ATNT Laboratories, India
Batch no. / Lot no.	202003021556
Order no.	Altromin Doc. No. 47764
Production date	02.03.2020
Expiry date	02.03.2021

Guaranteed nutritional values

% in air-dry substance	Value*	Tolerance**
Crude protein	19,2	16,8 – 21,6
Crude fat	4,1	3,1 – 6,1
Crude fibre	6,1	4,4 – 7,8
Crude ash	6,9	4,9 – 7,9
Moisture	11,3	< 12,4
NfE - Nitrogen free extracts	52,4	
Calcium	0,7	0,4 – 1,3
Phosphorus	0,5	0,2 – 0,8

* The producer guarantees that nutritional values of this batch are within the declared tolerance values
** Tolerances according to Annex IV of Regulation (EU) Nr.767/2009

Physical analysis

Pellet hardness kg/cm² - Kahl	22

Sensory evaluation

Olfactory	ok
Visual	ok

This product is compliant with the specifications and quality requirements of Altromin and therefore has been approved for delivery.

Accepted and released for use 12.05.20

Date: April 08th 2020

Hans-Leopold Altrogge
QA-Manager

This is a computer printout and has therefore not been signed and dated by hand

ANNEXURE 2 (Contd…). CONTAMINANT ANALYSIS TEST REPORT OF FEED

Nr.: QA - 72-1
Aufbewahrungsdauer
15 Jahre nach Erstellen

Altromin Spezialfutter GmbH & Co. KG
Im Seelenkamp 20
D-32791 Lage
Tel. +49 (0)5232 / 6088-0
Fax +49 (0)5232 / 6088-20

altromin

Producer Certificate

Description	1324 Maintenance diet for rats and mice
Customer	ATNT Laboratories, India
Batch no. / Lot no.	202003021556
Production date	02.03.2020
Expiry date	02.03.2021

Guaranteed diet status:

Aflatoxins	
Aflatoxin B1	< 2.5 µg/kg
Aflatoxin B2	< 0.6 µg/kg
Aflatoxin G1	< 2.5 µg/kg
Aflatoxin G2	< 0.6 µg/kg
Sum B1, B2, G1, G2	below detection limit
Heavy metals	
Lead (Pb)	< 1.00 mg/kg
Cadmium (Cd)	< 0.20 mg/kg
Mercury (Hg)	< 0.05 mg/kg
Arsenic (As)	< 1.00 mg/kg
Polychlorinated Biphenyls	
PCB	below detection limit
Pesticides and residuals	
Chlorpyriphos-methyl	< 0.100 mg/kg
Ethoxyquin	< 5.000 mg/kg
Piperonylbutoxid	< 0.500 mg/kg
Pirimiphos-methyl	< 0.500 mg/kg
all screened substances not mentioned are usually below detection limit (see attached list)	
Microbiological status	
Total aerobic count	< 10^5 cfu/g
Yeasts	< 10^2 cfu/g
Moulds	< 10^2 cfu/g
E. coli	< 10^1 cfu/g
Salmonella in 25 g	not detectable

Accepted and released for use 13.05.20

Hans-Leopold Altrogge
(Quality Manager)

Date: April 08th 2020

This is a computer printout and has therefore not been signed and dated by hand.

ANNEXURE 3. CONTAMINANT ANALYSIS TEST REPORT OF WATER

IADFAC Laboratories Pvt. Ltd.

INSTITUTE FOR ANALYSIS OF DAIRY,FOOD & CULTURES.
#8, Siddhi Vinayaka Complex, Nagarabhavi 2nd Stage,2nd Block
Near BDA Complex, 80 Feet Ring Road, Bangalore-560 072
Ph:+91-80-2318 6906 to 10, Cell : +91 8152881444/8152881222
Mail: accounts@iadfac.com/bd@iadfac.com/qa@iadfac.com

CERTIFICATE OF ANALYSIS

BOOKING NO. : 0010
CERTIFICATE NO. : 0010/2020-2021

NAME OF MANUFACTURER/PARTY : BIONEEDS INDIA PRIVATE LIMITED
Devarahosahalli ,Sompura Hobali, Nelamangla Taluk,
Bangalore Rural Dist,
BANGALORE - 562111 KARNATAKA

1. MFG. LIC. NO.	: NM		
2. REFERENCE NO.	: NM	3. DATE	: 06/05/2020
4. DATE OF RECEIPT	: 06/05/2020	5. NAME OF SAMPLE	: Drinking Water (R O Water)

6. DETAILS OF RAW MATERIAL / FINAL PRODUCTS (In Bulk/Finished Pack)

(A) MANUFACTURER NAME	: NM	(B) BATCH NO.	: NM
(C) BATCH SIZE	: NM	(D) DATE OF MFG.	: NM
(E) SAMPLE QUANTITY	: 5Lx1 Can	(F) DATE OF EXPIRY	: NM
(G) PACKING	: Plastic Bottle	(H) STARTING DATE	: 08/05/2020
(I) SEALED	: Sealed	(J) ENDING DATE :	: 19/05/2020
(K) BRAND NAME	: NM	(L) SAMPLING PROTOCOL	: NA
(M) DATE OF SAMPLING /SAMPLE COLLECTION	: 06/05/2020	(N) REPORT GEN. DATE	: 19/05/2020

Specification as per IS 10500:2012

SR	TEST NAME	UNIT	RESULT	ACCEPTABLE LIMIT	PERMISSIBLE LIMIT	METHOD OF TEST
1	CHEMICAL TESTING Water, Residues in Water					
1	Colour	CU	<1	5 Max	15 Max	IS 3025 (Part-4) : 1983
2	Odour	-	Agreeable	Agreeable	Agreeable	IS 3025 (Part-5) : 2018
3	pH Value	-	6.6	6.5-8.5	No Relaxation	IS 3025 (Part-11) : 1983
4	Taste	-	Agreeable	Agreeable	Agreeable	IS 3025 (Part-7&Part-8) : 2017
5	Turbidity (as NTU)	-	<1	1 Max	5 Max	IS 3025 (Part-10) : 1984
6	Total Dissolved Solids	mg/l	23	500 Max	2000 Max	IS 3025 (Part-16) : 1984
7	Aluminium (as Al)	mg/l	<0.02	0.03 Max	0.2 Max	IS 3025 (Part-55) : 2003
8	Boron (as B)	mg/l	<0.1	0.5 Max	2.4 Max	IS 3025 (Part-57) : 2005
9	Calcium (as Ca)	mg/l	1	75 Max	200 Max	IS 3025 (Part-40) : 1991
10	Chloride (as Cl)	mg/l	2	250 Max	1000 Max	IS 3025 (Part-32) : 1988
11	Copper (as Cu)	mg/l	<0.05	0.05 Max	1.5 Max	IS 3025 (Part-42) : 1992
12	Fluoride (as F)	mg/l	<0.1	1.0 Max	1.5 Max	IS 3025 (Part-60) : 2013
13	Free residual Chlorine	mg/l	<0.1	0.2 Min	1.0 Max	IS 3025 (Part-26) : 1986
14	Iron (as Fe)	mg/l	<0.05	1.0 Max	No Relaxation	IS 3025 (Part-53) : 2003
15	Magnesium (as Mg)	mg/l	<1	30 Max	100 Max	IS 3025 (Part-46) : 1994
16	Manganese (as Mn)	mg/l	<0.1	0.1 Max	0.3 Max	IS 3025 (Part-59) : 2006

Remarks : Accepted and released for use 21/05/2020

For IADFAC Laboratories Pvt. Ltd.
Karun
Authorised Signatory
Karunakara A.C. (ID No-132)
Senior Manager-Chemical

CONTD. ON NEXT PAGE.....

AUTHORISED SIGNATORY

Note :

1.The results listed,refer only to the samples analysed & applicable parameters, Endorsement products is neither inferred nor implied.
2.Total liability of our institute is limited to the invoiced amount.
3. The report cannot be reproduced, completely or in part, in any form of media(including print) without on explicit written permission from IADFAC Lab.
4. Sample drawn and submitted by the party for Analysis unless otherwise stated.
5. Analysed Food samples are destroyed within one month. Analysed Packaged Drinking Water samples destroyed after 3 months.

ANNEXURE 3 (Contd…). CONTAMINANT ANALYSIS TEST REPORT OF WATER

IADFAC Laboratories Pvt. Ltd.

INSTITUTE FOR ANALYSIS OF DAIRY,FOOD & CULTURES.
#8, Siddhi Vinayaka Complex, Nagarabhavi 2nd Stage,2nd Block
Near BDA Complex, 80 Feet Ring Road, Bangalore-560 072
Ph:+91-80-2318 6906 to 10, Cell : +91 8152881444/8152881222
Mail: accounts@iadfac.com/bd@iadfac.com/qa@iadfac.com

CERTIFICATE OF ANALYSIS

BOOKING NO. : 0010
CERTIFICATE NO. : 0010/2020-2021

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Devarahosahalli ,Sompura Hobali, Nelamangla Taluk,
Bangalore Rural Dist,
BANGALORE - 562111 KARNATAKA

1. MFG. LIC. NO. : NM
2. REFERENCE NO. : NM
3. DATE : 06/05/2020
4. DATE OF RECEIPT : 06/05/2020
5. NAME OF SAMPLE : Drinking Water (R O Water)

6. DETAILS OF RAW MATERIAL / FINAL PRODUCTS (In Bulk/Finished Pack)

(A) MANUFACTURER NAME	: NM	(B) BATCH NO.	: NM
(C) BATCH SIZE	: NM	(D) DATE OF MFG.	: NM
(E) SAMPLE QUANTITY	: 5Lx1 Can	(F) DATE OF EXPIRY	: NM
(G) PACKING	: Plastic Bottle	(H) STARTING DATE	: 08/05/2020
(I) SEALED	: Sealed	(J) ENDING DATE :	: 19/05/2020
(K) BRAND NAME	: NM	(L) SAMPLING PROTOCOL	: NA
(M) DATE OF SAMPLING /SAMPLE COLLECTION	: 06/05/2020	(N) REPORT GEN. DATE	: 19/05/2020

Specification as per IS 10500:2012

SR	TEST NAME	UNIT	RESULT	ACCEPTABLE LIMIT	PERMISSIBLE LIMIT	METHOD OF TEST
17	Nitrate (as NO3)	mg/l	1.16	45 Max	No Relaxation	IS 3025 (Part-34) : 1988
18	Selenium (as Se)	mg/l	<0.01	0.01 Max	No Relaxation	IS 3025 (Part-56) : 2003
19	Sulphate (as SO4)	mg/l	<1	200 Max	400 Max	IS 3025 (Part-24) : 1986
20	Total Alkalinity as calcium carbonate	mg/l	8.0	200 Max	600 Max	IS 3025 (Part-23) : 1986
21	Total Hardness (as CaCO3)	mg/l	4	200 Max	600 Max	IS 3025 (Part-21) : 2009
22	Cadmium (as Cd)	mg/l	<0.003	0.003 Max	No Relaxation	IS 3025 (Part-41) : 1992
23	Lead (as Pb)	mg/l	<0.01	0.01 Max	No Relaxation	IS 3025 (Part-47) : 1994
24	Mercury (as Hg)	mg/l	<0.001	0.001 Max	No Relaxation	IS 3025 (Part-48) : 1994
25	Total Arsenic (as As)	mg/l	<0.01	0.01 Max	No Relaxation	IS 3025 (Part-37) : 1988
26	Total Chromium (as Cr)	mg/l	<0.05	0.05 Max	No Relaxation	Annexure- J of IS 13428 : 2005
	Pesticide residues					
1	Endosulfan					
a	Alpha Endosulfan	µg/l	<0.01	0.4 Max	No Relaxation	FSSAI Manual of water 2016
b	Beta Endosulfan	µg/l	<0.01	0.4 Max	No Relaxation	FSSAI Manual of water 2016
c	Endosulfan sulphate	µg/l	<0.01	0.4 Max	No Relaxation	FSSAI Manual of water 2016
2	Ethion	µg/l	<0.01	3 Max	No Relaxation	FSSAI Manual of water 2016

Remarks : Accepted and released for use 21/05/2020

For IADFAC Laboratories Pvt. Ltd.
Authorised Signatory
Karunakara A.C. (ID No-132)
Senior Manager-Chemical

CONTD. ON NEXT PAGE.....

AUTHORISED SIGNATORY

Note :
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4. Sample drawn and submitted by the party for Analysis unless otherwise stated.
5. Analysed Food samples are destroyed within one month. Analysed Packaged Drinking Water samples destroyed after 3 months.

ANNEXURE 3 (Contd…). CONTAMINANT ANALYSIS TEST REPORT OF WATER

IADFAC Laboratories Pvt. Ltd.

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Devarahosahalli ,Sompura Hobali, Nelamangla Taluk,
Bangalore Rural Dist,
BANGALORE - 562111 KARNATAKA

1. MFG. LIC. NO.	: NM		
2. REFERENCE NO.	: NM	3. DATE	: 06/05/2020
4. DATE OF RECEIPT	: 06/05/2020	5. NAME OF SAMPLE	: Drinking Water (R O Water)

6. DETAILS OF RAW MATERIAL / FINAL PRODUCTS (In Bulk/Finished Pack)

(A) MANUFACTURER NAME	: NM	(B) BATCH NO.	: NM
(C) BATCH SIZE	: NM	(D) DATE OF MFG.	: NM
(E) SAMPLE QUANTITY	: 5Lx1 Can	(F) DATE OF EXPIRY	: NM
(G) PACKING	: Plastic Bottle	(H) STARTING DATE	: 08/05/2020
(I) SEALED	: Sealed	(J) ENDING DATE :	: 19/05/2020
(K) BRAND NAME	: NM	(L) SAMPLING PROTOCOL	: NA
(M) DATE OF SAMPLING /SAMPLE COLLECTION	: 06/05/2020	(N) REPORT GEN. DATE	: 19/05/2020

Specification as per IS 10500:2012

SR	TEST NAME	UNIT	RESULT	ACCEPTABLE LIMIT	PERMISSIBLE LIMIT	METHOD OF TEST
3	Monocrotophos	µg/l	<0.01	1 Max	No Relaxation	FSSAI Manual of water 2016
--	--	--	-- End of Report --	--	--	--

Remarks : Accepted & released for use 21/05/2020

For IADFAC Laboratories Pvt. Ltd.
Authorised Signatory
Karunakara A.C. (ID No-132)
Senior Manager-Chemical

AUTHORISED SIGNATORY

Note :

1. The results listed,refer only to the samples analysed & applicable parameters, Endorsement products is neither inferred nor implied.
2. Total liability of our institute is limited to the invoiced amount.
3. The report cannot be reproduced, completely or in part, in any form of media(including print) without on explicit written permission from IADFAC Lab.
4. Sample drawn and submitted by the party for Analysis unless otherwise stated.
5. Analysed Food samples are destroyed within one month. Analysed Packaged Drinking Water samples destroyed after 3 months.

Page 3 of 3

ANNEXURE 4. CONTAMINANT ANALYSIS TEST REPORT OF BEDDING MATERIAL

IADFAC Laboratories Pvt. Ltd.

INSTITUTE FOR ANALYSIS OF DAIRY,FOOD & CULTURES.
#8, Siddhi Vinayaka Complex, Nagarabhavi 2nd Stage,2nd Block
Near BDA Complex, 80 Feet Ring Road, Bangalore-560 072
Ph:+91-80-2318 6906 to 10, Cell : +91 8152881444/8152881222
Mail: accounts@iadfac.com/bd@iadfac.com/qa@iadfac.com

CERTIFICATE OF ANALYSIS

BOOKING NO. 0011
CERTIFICATE NO. : 0011/2020-2021

NAME OF MANUFACTURER/PARTY : BIONEEDS INDIA PRIVATE LIMITED
Devarahosahalli ,Sompura Hobali, Nelamangla Taluk,
Bangalore Rural Dist,
BANGALORE - 562111 KARNATAKA

1.MFG. LIC. NO.	NM	4. OTHER REFERENCE NO	NM
2. REFERENCE NO.	NM	5. DATE OF RECEIPT	06/05/2020
3. DATE	06/05/2020	6. NAME OF SAMPLE	Paddy husk

7. DETAILS OF RAW MATERIAL / FINAL PRODUCTS (In Bulk/Finished Pack)

(A) BATCH NO.	NM	(H) SEALED	Sealed
(B) BATCH SIZE	NM	(I) STARTING DATE	08/05/2020
(C) DATE OF MFG.	NM	(J) ENDING DATE :	19/05/2020
(D) DATE OF EXPIRY	NM	(K) BRAND NAME	NM
(E) SAMPLE QUANTITY	1 kg	(L) SAMPLING PROTOCOL	NA
(F) MFG NAME	NM	(M) DATE OF SAMPLING /SAMPLE COLLECTION	06/05/2020
(G) PACKING	Zip lock cover	(N) REPORT GEN. DATE	19/05/2020

SR	TEST NAME	UNIT	RESULT	SPECIFICATIONS	METHOD OF TEST
I	CHEMICAL TESTING				
	Animal Food & Feed				
	Heavy Metals				
1	Arsenic	mg/kg	<0.1	-	AOAC 20th Edition 2016
2	Lead	mg/kg	<0.1	-	AOAC 20th Edition 2016
3	Cadmium	mg/kg	<0.1	-	AOAC 20th Edition 2016
4	Mercury	mg/kg	<0.1	-	AOAC 20th Edition 2016
	Chlorinated Hydrocarbons				
1	Hexachlorobenzene (HCB)	mg/kg	Not detected	-	AOAC 20th Edition 2016
2	Hexachlorocylcohexane (HCH)	mg/kg	Not detected	-	AOAC 20th Edition 2016
3	HCH (Lindane)	mg/kg	Not detected	-	AOAC 20th Edition 2016
4	Heptachlor & epoxide	mg/kg	Not detected	-	AOAC 20th Edition 2016
5	Chlordane	mg/kg	Not detected	-	AOAC 20th Edition 2016
6	Aldrin	mg/kg	Not detected	-	AOAC 20th Edition 2016
7	Dieldrin	mg/kg	Not detected	-	AOAC 20th Edition 2016
8	Endrin	mg/kg	Not detected	-	AOAC 20th Edition 2016
9	DDE	mg/kg	Not detected	-	AOAC 20th Edition 2016
10	DDD	mg/kg	Not detected	-	AOAC 20th Edition 2016

Remarks : Accepted and released for use 21/05/2020

For IADFAC Laboratories Pvt. Ltd.
Authorised Signatory
Karunakara A.C. (ID No-132)
Senior Manager-Chemical

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5. Analysed Food sample destroyed within one month. Analysed Packaged Drinking Water sample destroyed after 3 months.

ANNEXURE 4 (Contd…). CONTAMINANT ANALYSIS TEST REPORT OF BEDDING MATERIAL

IADFAC Laboratories Pvt. Ltd.

INSTITUTE FOR ANALYSIS OF DAIRY,FOOD & CULTURES.
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Bangalore Rural Dist,
BANGALORE - 562111 KARNATAKA

1.MFG. LIC. NO.	NM	4. OTHER REFERENCE NO	NM
2. REFERENCE NO.	NM	5. DATE OF RECEIPT	06/05/2020
3. DATE	06/05/2020	6. NAME OF SAMPLE	Paddy husk

7. DETAILS OF RAW MATERIAL / FINAL PRODUCTS (In Bulk/Finished Pack)

(A) BATCH NO.	NM	(H) SEALED	Sealed
(B) BATCH SIZE	NM	(I) STARTING DATE	08/05/2020
(C) DATE OF MFG.	NM	(J) ENDING DATE :	19/05/2020
(D) DATE OF EXPIRY	NM	(K) BRAND NAME	NM
(E) SAMPLE QUANTITY	1 kg	(L) SAMPLING PROTOCOL	NA
(F) MFG NAME	NM	(M) DATE OF SAMPLING /SAMPLE COLLECTION	06/05/2020
(G) PACKING	Zip lock cover	(N) REPORT GEN. DATE	19/05/2020

SR	TEST NAME	UNIT	RESULT	SPECIFICATIONS	METHOD OF TEST
11	DDT	mg/kg	Not detected	-	AOAC 20th Edition 2016
12	Endosulfan	mg/kg	Not detected	-	AOAC 20th Edition 2016
13	Endosulfan Sulphate	mg/kg	Not detected	-	AOAC 20th Edition 2016
14	Phosphoric Acid Esters	mg/kg	Not detected	-	AOAC 20th Edition 2016
15	Malathion	mg/kg	Not detected	-	AOAC 20th Edition 2016
16	Fenitrothion	mg/kg	Not detected	-	AOAC 20th Edition 2016
17	Pirimiphos(-methyl)	mg/kg	Not detected	-	AOAC 20th Edition 2016
18	Chlorpyiphos (-methyl)	mg/kg	Not detected	-	AOAC 20th Edition 2016
19	All other Phosphates	mg/kg	Not detected	-	AOAC 20th Edition 2016
20	Polychlorinated Biphenyls (PCB)	mg/kg	Not detected	-	AOAC 20th Edition 2016
	Mycotoxins				
1	Aflatoxin B1	µg/kg	Not detected	-	AOAC 20th Edition 2016
2	Aflatoxin B2	µg/kg	Not detected	-	AOAC 20th Edition 2016
3	Aflatoxin G1	µg/kg	Not detected	-	AOAC 20th Edition 2016
4	Aflatoxin G2	µg/kg	Not detected	-	AOAC 20th Edition 2016
5	Zearalenone	µg/kg	Not detected	-	AOAC 20th Edition 2016
6	Ochratoxin A	µg/kg	Not detected	-	AOAC 20th Edition 2016
	Nitrosamines				
1	Nitrosodiethylamine	µg/kg	Not detected	-	AOAC 20th Edition 2016

Remarks :
Accepted and released for use on 21/05/2020

For IADFAC Laboratories Pvt. Ltd.
Authorised Signatory
Karunakara A.C. (ID No-132)
Senior Manager-Chemical

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4. Sample drawn and submitted by the party for Analysis unless otherwise stated.
5. Analysed Food sample destroyed within one month. Analysed Packaged Drinking Water sample destroyed after 3 months.

ANNEXURE 4 (Contd…). CONTAMINANT ANALYSIS TEST REPORT OF BEDDING MATERIAL

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IADFAC Laboratories Pvt. Ltd.

CERTIFICATE OF ANALYSIS

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1.MFG. LIC. NO.	NM	4. OTHER REFERENCE NO	NM
2. REFERENCE NO.	NM	5. DATE OF RECEIPT	06/05/2020
3. DATE	06/05/2020	6. NAME OF SAMPLE	Paddy husk

7. DETAILS OF RAW MATERIAL / FINAL PRODUCTS (In Bulk/Finished Pack)

(A) BATCH NO.	NM	(H) SEALED	Sealed
(B) BATCH SIZE	NM	(I) STARTING DATE	08/05/2020
(C) DATE OF MFG.	NM	(J) ENDING DATE :	19/05/2020
(D) DATE OF EXPIRY	NM	(K) BRAND NAME	NM
(E) SAMPLE QUANTITY	1 kg	(L) SAMPLING PROTOCOL	NA
(F) MFG NAME	NM	(M) DATE OF SAMPLING /SAMPLE COLLECTION	06/05/2020
(G) PACKING	Zip lock cover	(N) REPORT GEN. DATE	19/05/2020

SR	TEST NAME	UNIT	RESULT	SPECIFICATIONS	METHOD OF TEST
2	Nitrosodimethylamine	µg/kg	Not detected	-	AOAC 20th Edition 2016
--	--	--	-- End of Report --	--	--

Remarks :
Accepted and released for use
21/05/2020

For IADFAC Laboratories Pvt. Ltd.
Authorised Signatory
Karunakara A.C. (ID No-132)
Senior Manager-Chemical
AUTHORISED SIGNATORY

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Page 3 of 3

ANNEXURE 5. GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS (GHS)

Acute Inhalation Classifications

Exposure	Category 1	Category 2	Category 3	Category 4	Category 5
Dust and Mists (mg/L)	ATE ≤ 0.05	0.05 <ATE ≤ 0.5	0.5 <ATE ≤ 1.0	1.0 <ATE ≤ 5.0	See Note : (a)

ATE: Acute Toxicity Estimate Values

Note:

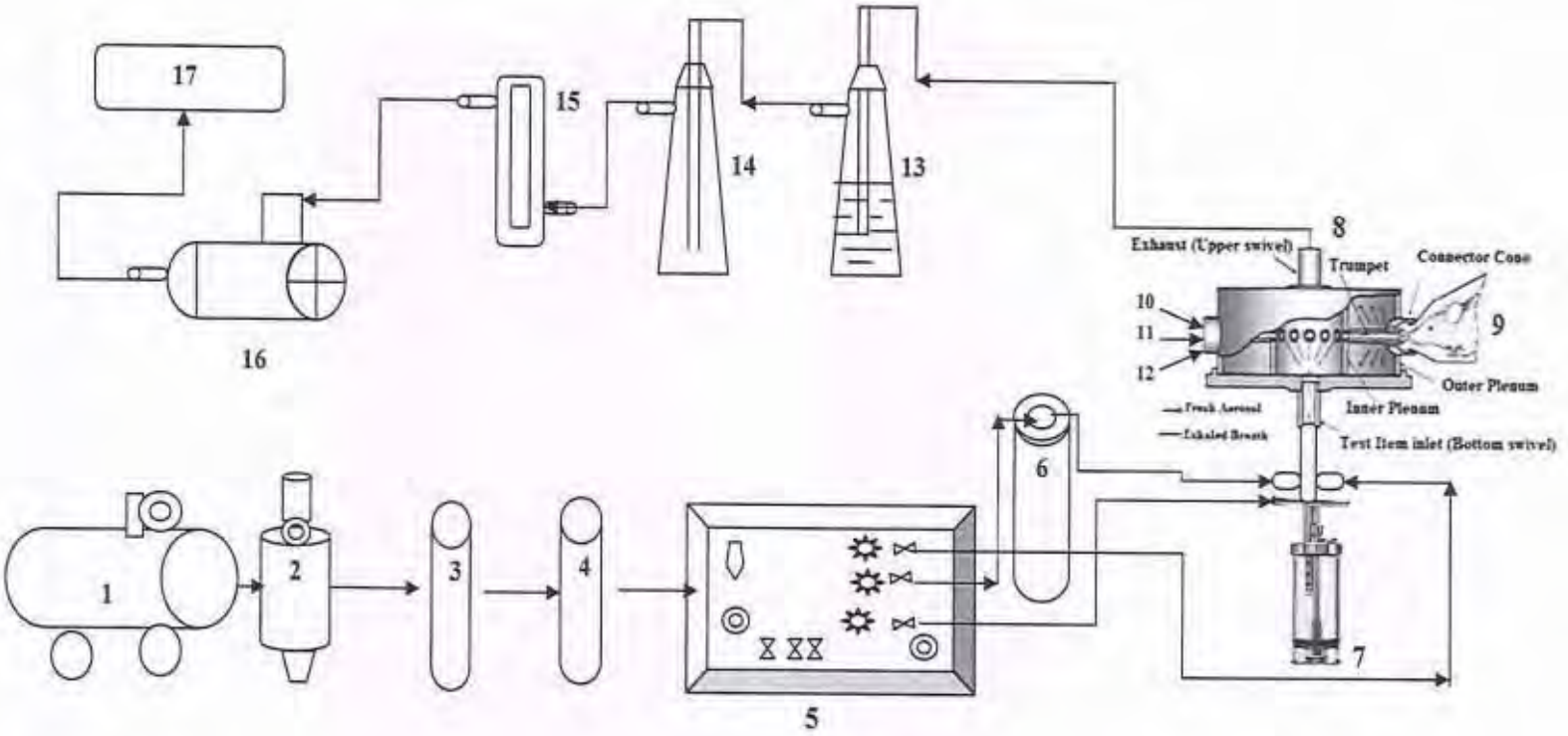
a) Criteria for Category 5 are intended to enable the identification of substances which are of relatively low acute toxicity hazard but which under certain circumstances may present a danger to vulnerable populations. These substances are anticipated to have on oral or dermal LD_{50} in the range of 2000-5000 mg/kg body weight and equivalent doses for inhalation. The specific criteria for Category 5 are:

i) The test item is classified in this category if reliable evidence is already available that indicates the LC_{50} to be in the range of Category 5 values or other animal studies or toxic effects in humans indicate a concern for human health of an acute nature.

ii) The test item is classified in this category, through extrapolation, estimation or measurement of data, if assignment to a more hazardous category is not warranted, and

- Reliable information is available indicating significant toxic effects in humans; or
- Any mortality is observed when tested up to Category 4 values by the oral, inhalation or dermal routes; or
- Where expert judgment confirms significant clinical signs of toxicity, when tested up to Category 4 values, except for diarrhoea, piloerection or an ungroomed appearance; or
- Where expert judgment confirms reliable information indicating the potential for significant acute effects from other animal studies.

ANNEXURE 6. LAYOUT OF FLOW- PAST, NOSE-ONLY DYNAMIC INHALATION EXPOSURE UNIT FOR LIQUID AEROSOL



Key:	1	:	Air Compressor	7	:	Collision Nebulizer	13	: 1% w/v NaOH
	2	:	Pressure Regulating Valve	8	:	Nose only Inhalation Chamber (CH Technologies, USA)	14	: Absorbent Cotton
	3	:	Charcoal Column	9	:	Animal Restrainer	15	: Rotameter
	4	:	Silica gel Column	10	:	Temperature, RH, Carbon dioxide and Oxygen content	16	: Vacuum Pump
	5	:	Control Panel	11	:	Breathing Zone Concentration (Actual Concentration)	17	: Fume Hood
	6	:	Humidifier, if required	12	:	Particle Size Measurement		

ANNEXURE 7. GLP CERTIFICATE

सत्यमेव जयते

National Good Laboratory Practice (GLP) Compliance Monitoring Authority (NGCMA)
Department of Science and Technology
GOVERNMENT OF INDIA

Certificate of GLP Compliance

This is to certify that

Bioneeds India Private Limited
Devarahosahally, Sompura Hobli, Nelamangala Taluk
Bengaluru Rural District - 562111, Karnataka (India)

is a GLP certified test facility in compliance with the NGCMA's Document No. GLP-101 "Terms & Conditions of NGCMA for obtaining and maintaining GLP certification by a test facility" and OECD Principles of GLP.

The test facility conducts the below-mentioned tests/ studies:

- **Physical-chemical Testing (Including Five Batch Analysis)**
- **Toxicity Studies**
- **Mutagenicity Studies**
- **Environmental Toxicity Studies on Aquatic and Terrestrial Organisms**
- **Studies on Behaviour in Water, Soil and Air; Bioaccumulation**
- **Residue Studies**
- **Analytical and Clinical Chemistry Testing**
- **Others**

The specific areas of expertise, test items and test systems are listed in the annexure overleaf.

Validity: September 23, 2020 – September 22, 2023

Certificate No. : GLP/C-153/2020
Issue Date : 13-10-2020

(Dr. Neeraj Sharma)
Head, NGCMA

ANNEXURE 7 (Contd…). GLP CERTIFICATE

National GLP Compliance Monitoring Authority (NGCMA)

Annexure to Certificate of GLP Compliance No. GLP/C-153/2020

Areas of Expertise:

- **Physical-chemical Testing (Including Five Batch Analysis)**
- **Toxicity Studies**
  - Acute Toxicity
  - Developmental and Reproductive Toxicity
  - Eye Irritation/ Corrosion (*in vitro* and *in vivo*)
  - Guinea Pig Maximization
  - Immunogenicity
  - Inhalation Toxicity
  - Local Lymph Node Assay (LLNA)
  - Local Tolerance
  - Neurotoxicity
  - Phototoxicity
  - Pyrogen Test
  - Repeated Dose Toxicity
  - Skin Irritation/ Corrosion (*in vitro* and *in vivo*)
  - Skin Sensitization (*in vitro* and *in vivo*)
- **Mutagenicity Studies**
  - 3T3 NRU Assay (*in vitro*)
  - Bacterial Reverse Mutation (AMES) Test
  - Cell Gene Mutation Test (*in vitro and in vivo*)
  - Chromosomal Aberration Test (*in vitro* and *in vivo*)
  - Comet Assay
  - Cytotoxicity (*in vitro*)
  - Micronucleus Test (*in vitro* and *in vivo*)
  - Mouse Lymphoma Assay (MLA)
  - MTT Assay
- **Environmental Toxicity Studies on Aquatic and Terrestrial Organisms**
- **Studies on Behaviour in Water, Soil and Air; bioaccumulation**
- **Residue Studies**
- **Analytical and Clinical Chemistry Testing**
- **Others**
  - ADME Studies
  - Bioanalysis
  - Biocompatibility Studies
  - Drug Metabolism & Pharmacokinetic (DMPK)
  - Hemocompatibility Studies
  - Implantation Studies
  - In chemico Skin Sensitization: Direct Peptide Reactivity Assay
  - Maximum Tolerated Dose (MTD) Studies
  - Method Development
  - Method Validation
  - Skin Absorption (*in vitro*)

Test Item(s): Agrochemicals, Cosmetics Products, Feed Additives, Food Additives, Industrial chemicals, Medical Devices (*Applicable only for Bio-compatibility, not applicable for Batch Release parameters required as per MDR, 2017*) and Pharmaceuticals (Human and Veterinary)

Test System(s): Algae, Bovine, Cell lines, Chicken, Collembolan, Crop plant seeds, Cyanobacteria, Daphnia, Diatoms, Earthworm, *E-Coli*, Fish, Guinea Pigs, Hamsters, Honeybees, Human Cavader Skin, Human Lymphocytes, Japanese quail, Lemna, Mallard duck, Mice, Pigeon, Predatory Mites, Rabbit, Rat, *Salmonella typhimurium*, Silkworm and Tissue Culture.

(Dr. Neeraj Sharma)
Head, NGCMA